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Life History and Abundance of *Mysis relicta* in Lake Michigan[1]

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MORGAN, M. D., AND A. M. BEETON. 1978. Life history and abundance of *Mysis relicta* in Lake Michigan. J. Fish. Res. Board Can. 35: 1165–1170.

Mysis relicta was sampled in Lake Michigan approximately monthly with an epibenthic sled, August 1975–July 1976. Total numbers ranged from a low of 23 m^{-2} in February to a high of 337 m^{-2} in May and averaged 188 m^{-2}. It was possible to partition the population into five instars. Major peaks in proportions of first instar individuals occurred in March, July, and November. It took about 1 yr for first instar individuals to reach the fourth instar. Growth averaged a little less than 1 mm/mo. Males and females reached maturity and bred in the fourth instar, so females produced their first brood at 1 yr of age. Males died at this time, but females were found to molt to the fifth instar, mate, and produce a second brood approximately 4 mo later.

Key words: Mysis relicta, Lake Michigan, life history, abundance

MORGAN, M. D., AND A. M. BEETON. 1978. Life history and abundance of *Mysis relicta* in Lake Michigan. J. Fish. Res. Board Can. 35: 1165–1170.

Nous avons prélevé à peu près mensuellement des échantillons de *Mysis relicta* dans le lac Michigan à l'aide d'un traîneau épibenthique, d'août 1975 à juillet 1976. Le nombre total d'individus varie d'un minimum de 23 m^{-2} en février à un maximum de 337 m^{-2} en mai et est de 188 m^{-2} en moyenne. Il a été possible de diviser la population en cinq instars. Les pics les plus importants dans les proportions d'individus de premier instar se produisent en mars, juillet et novembre. Les individus de premier instar prennent environ 1 an à atteindre le quatrième instar. La croissance est en moyenne un peu moins de 1 mm/mo. Les mâles et les femelles atteignent la maturité et se reproduisent au quatrième instar, de sorte que les femelles produisent leur première portée à l'âge d'un an. Les mâles meurent à ce moment-là, mais nous avons constaté que les femelles muent et passent au cinquième instar, s'accouplent et produisent une seconde portée environ 4 mo plus tard.

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SEVERAL factors combine to make *Mysis relicta* a key species in the ecology of the Great Lakes. It is large, attaining lengths >2 cm in Lake Michigan, and can feed on zooplankton, phytoplankton, and detritus (Tattersall and Tattersall 1951; Lasenby and Langford 1973). Such feeding habits may have a significant effect on the structure of both the phytoplankton and zooplankton communities. In fact, in lakes where *M. relicta* has been introduced, dramatic shifts in the phytoplankton and zooplankton communities have been observed (Zyblut 1970; Richards et al. 1975). *Mysis* is also highly mobile, undergoing extensive diel vertical migrations (Beeton 1960). The impact of these migrations on nutrient transport from the sediment–water interface, where the animal spends most of its time during the day, to the region of the thermocline, where it migrates at night (Beeton 1960; Teraguchi 1969), is still unresolved, but potentially very great. Further, the importance of *M. relicta* in the diets of important commercial and sport fish in the Great Lakes is well documented (Schneberger 1936; Van Oosten and Deason 1937; Wells and Beeton 1963).

Consequently, knowledge of the biology of *M. relicta* is important to an understanding of the entire Great Lakes ecosystem. Yet, *Mysis* population dynamics are poorly understood. Individuals may be benthic and/or planktonic depending on such variables as light intensity, depth, and age (Beeton 1959, 1960; McWilliams 1970) and collections of representative population estimates are difficult. As a result, *Mysis* has been largely ignored in most zooplankton and benthic investigations in the Great Lakes.

Work by Juday and Birge (1927) and Larkin (1948) represents the earliest attempts to study the population dynamics of *M. relicta* in North America. More recently, a number of studies have been conducted on lakes in Canada and the United States (Brownell 1970; McWilliams 1970; Lasenby and Langford 1972; Reynolds and DeGraeve 1972; Carpenter et al. 1974). However, before *Mysis* population dynamics can be satisfactorily worked out, year-round samples and at least representative, if not quantitative, population estimates must be obtained. Few studies have been based on year-round samples, especially in the Great Lakes. This study was undertaken to provide information on *M. relicta* based on year-round sampling.

Materials and Methods

Samples were collected approximately monthly in Lake Michigan, August 1975 through July 1976 at a sampling location 30 km northeast of Milwaukee. The sounded depth ranged from 109 to 121 m. An epibenthic sled with a 1-m^2 mouth (no. 0 mesh) was the primary sampling device and was used only during the day, usually between 1000 and 1400 CST. Each tow was 3 min once the sled was on the bottom. Sampling was terminated by a messenger-activated release mechanism which closed the net. Sampling distance was measured by a flow meter. Three replicate tows were taken on all sampling dates except for December, February, and July when two tows were taken and November when only one tow was taken. All samples were preserved in 10% buffered formalin.

The in situ observations from a submarine at this depth during the day (Robertson et al. 1968) and the negative phototropism of this organism (Beeton 1959, 1960) indicate that most of the mysids would have been at or near the bottom during sampling. To verify this a number of vertical tows were made with a ¾ m diam net (no. 0 mesh). No mysids were collected 3 m or more above the bottom.

All samples were subsampled from 1/16 to 1/256 with a Folsom Plankton Splitter and counted. The Folsom Plankton Splitter has been used for mysids previously with an estimated error of 10% at the 95% confidence level (McWilliams 1970). Replicate samples were then pooled and further reduced to approximately 100 individuals for size, sex, and instar analysis.

Males were identified by the presence of the appendix masculina at the bases of the first antennae, or by the presence of enlarged fourth pleopods. Females were identified by the presence of a brood pouch. Individuals without either of the above characteristics were classified as sexually undifferentiated juveniles. Males were considered mature on the basis of complete development of the fourth pleopod (Tattersall and Tattersall 1951), and females were considered mature if they had fully developed brood pouches.

Total length of straightened animals was measured under a dissecting microscope to the nearest 0.5 mm from the tip of the rostrum to the apical cleft of the telson. Statoliths were dissected from the uropods and their diameter measured with an ocular micrometer for instar analysis.

Results

Mysids were grouped into five instars (Fig. 1) on the basis of statolith diameters. Statolith diameters from 59 molted exoskeletons obtained from laboratory cultures of *M. relicta* verified molts between the first and second, third and fourth, and fourth and fifth instars (N. Grossnickle, University of Wisconsin, Milwaukee, Wis., personal communication). Four exuvia were collected with statoliths of 0.114, 0.121, 0.157, and 0.164 mm, which may indicate the molt between the second and third instar, but these were too few to verify the molting length. These exuvia also suggested the possibility of a molt in the first instar group when statoliths were 0.05–0.057 mm. Such an additional molt is indicated in Fig. 1, but is not distinct enough to be clearly identified. Statolith measurements for Fig. 1 were accurate to only 0.01 mm. This may have been too coarse a measure to distinguish this instar.

Only members of the first instar could not be sexed and are referred to as juveniles. All fifth instar individuals were females, while the other instars consisted of both males and females. Males and females exhibited a similar range of statolith diameters. Statolith diameter within instars did not change seasonally. Also, male and female mysids were in the same ranges in total length for instars II and III. Females had a much greater range in length and the smallest female was larger than the smallest male in the instar IV group (Morgan 1976). Over the entire study period, the male/female ratio re-

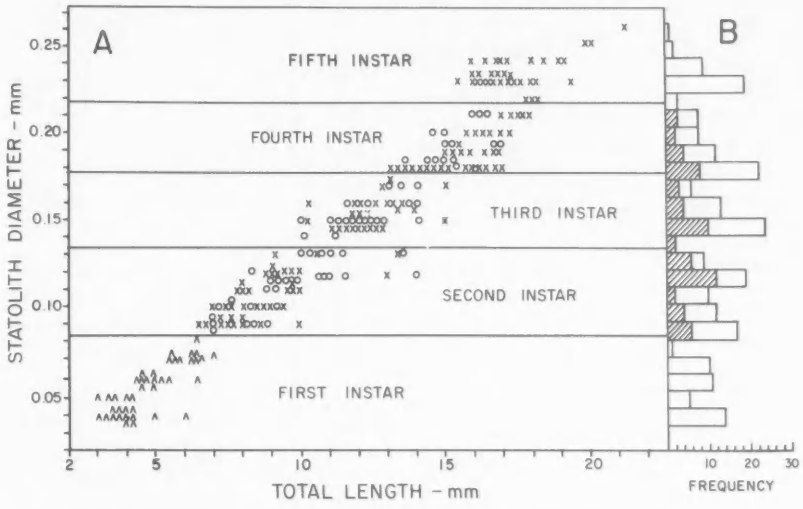

FIG. 1. Relationship of statolith diameter (mm) to total length (mm) for a population of *Mysis relicta* from Lake Michigan (A). Designation into proposed instars is based on frequency distribution of statolith sizes (B). X represents female, o male, and Λ sexually undifferentiated. Hatched bars are statolith size frequency for males, plain bar for all others.

mained very nearly 1:1, with females being slightly more abundant.

During the study no second instar and only a small percentage of the third instar individuals were mature (2.1% male, 2.6% female), while 64.8% of the fourth instar males and 84.3% of the fourth and 96.3% of the fifth instar females were mature. These data suggest that the molt between instar three and four, as determined by statolith analysis, was the time when both males and females become sexually mature. In addition, since females cannot molt while brooding young, the presence of sexually mature and breeding fifth instar females suggests a second brood may be produced.

The number of mysids captured over the sampling period averaged 188 m^{-2} and ranged from a maximum of 337 m^{-2} in May to a minimum of 23 m^{-2} in February. All instars except the fifth made up at least 10% of the population (Fig. 2) indicating that reproduction occurred more than once during the year. Three distinct peaks in percentage of juveniles occurred in March, July, and November. Consequently, most recruitment occurred at specific times and the population could be divided into distinct cohorts.

These cohorts were followed through their growth stages by tracing successive peaks in the later instars (Fig. 2). The March 1976 peak in abundance of juveniles (instar I) was followed by increased abundance of second instars in June. If we assume that this was the usual annual trend, then a similar cohort would have attained instar III status in October 1976. The October 1975 pulse in instar III was followed by a February 1976 increase in the IV instar. Similarly a July peak in instar I would have been followed by an October peak in instar II. Progression of this cohort into instar III was not apparent, however (Fig. 2). It may be that this peak in the third instar was missed due to lack of January samples when this peak could have occurred. A November peak in the first instar was small, but it was followed by slight increases in instar II in February and instar III in May. Too few individuals of instar V were taken to include in this analysis.

For each cohort, it would take slightly less than 1 yr to reach the fourth instar. Since females become sexually mature and breed in the fourth instar, it appears that a cohort produces its first offspring at 1 yr of age. Thus, the peak abundance of fourth instar individuals in February probably gave rise to the juveniles in March, and similarly, September and May peaks would give rise to the peaks in abundance of juveniles in November and July.

The growth rate of individuals for a cohort, which probably entered the population in March 1975, was determined by plotting the mode of the total length for each sampling date (Fig. 3) starting with August 1975 when modal length was 8 mm. The growth rate of this cohort before sampling in August 1975 could not be estimated. However, the March 1976 cohort started out at approximately 4 mm and it was assumed this was true for March 1975. For comparative purposes, growth rate estimates by Beeton (unpublished Lake Michigan data, 1954–55), Lasenby and Langford (1972), and Reynolds and DeGraeve (1972) are also included in the figure. Growth was a little less than 1 mm/mo. The growth rate estimates in all of these studies are closely similar.

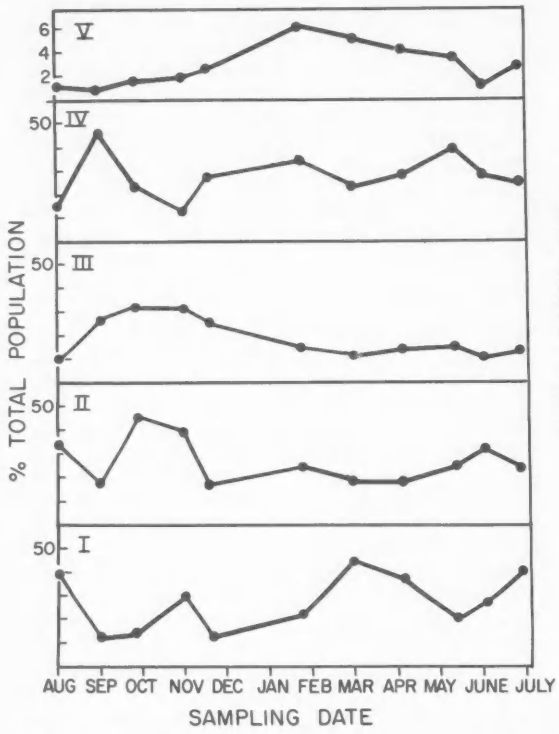

FIG. 2. Proportion of the total population present in each instar over the sampling period. Roman numerals indicate instars. Note change of scale for instar V. Short vertical lines indicate middle of month.

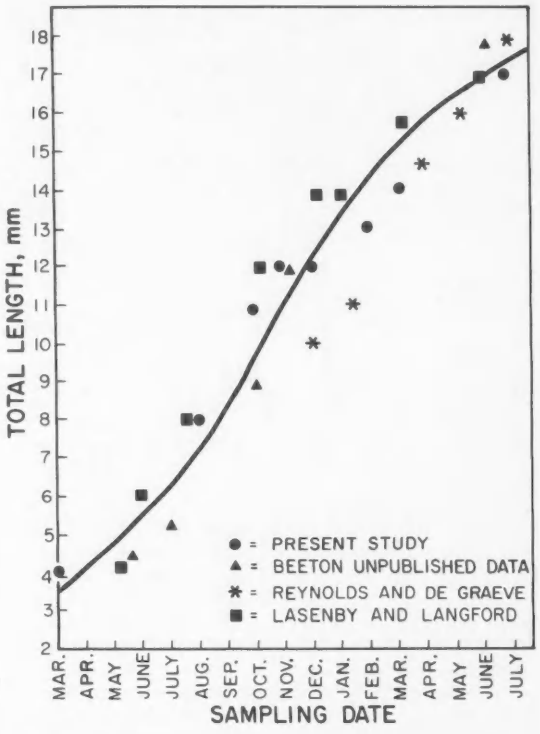
TOTAL LENGTH, mm
● = PRESENT STUDY
▲ = BEETON UNPUBLISHED DATA
✱ = REYNOLDS AND DE GRAEVE
■ = LASENBY AND LANGFORD
MAR. APR. MAY JUNE JULY AUG. SEP. OCT. NOV. DEC. JAN. FEB. MAR. APR. MAY JUNE JULY
SAMPLING DATE

FIG. 3. Estimated growth rate of *Mysis relicta* in Lake Michigan (present study; Beeton unpublished data; Reynolds and DeGraeve 1972) and Stony Lake (Lasenby and Langford 1972). Short vertical lines indicate middle of month.

The approximate mean total lengths of instars in Fig. 1 were as follows: I, 4.5 mm; II, 9 mm; III, 12 mm; IV, 16 mm. According to the growth curve (Fig. 3) it would take about 3 mo for a 9-mm individual to grow to 12 mm and a little more than 3 additional months to attain a length of 16 mm. It took about 3 mo for a cohort, as indicated by a peak in abundance (Fig. 2), to change from one instar to the next. The growth curve indicates that it would take about 5 mo for a 4.5-mm individual to grow to 9 mm. Consequently, this is not in accordance with the interpretation of the data from Fig. 2, which indicates 3 mo to progress from instar I to instar II. The frequency distribution of statolith diameters (Fig. 1) indicates the possibility of two instars making up what is named the first instar. If this is the situation the true first instar would have a mean length of about 4 mm, grow to about 5.5 mm in about 2½ mo, (instar II) and attain a length of 9 mm in 3 more months.

The production of two groups of young by a cohort, one at 12 mo and one at 16 mo, leads to a complex population structure with six distinct cohorts represented in the population during any one year. Figure 4 illustrates how this structure would develop. Starting with a group of juveniles in March of year I (cohort 1), a major production of young is observed in March of year II (cohort 2). However, some females survive to instar V and produce a second group of young in July of year II (cohort 3). Cohort 2 follows the same pattern as cohort 1, while cohort 3 produces its first brood in July (cohort 5) and a second brood in November (cohort 6) in year III. Cohort 5 follows a pattern similar to cohort 3, but cohort 6 produces young in November of year IV (cohort 9) and March of year V (cohort 10). With the production of cohort 10, the cycle is completed, and a stable population structure is obtained. This population structure then accounts for the periodic release of young.

Discussion

It was assumed that the same population of mysids was being sampled to be able to make valid estimates of growth and determine the structure of the population. If significant migrations of mysids into or out of the sampling area were to occur the data could be badly skewed. The only evidence that such migrations might

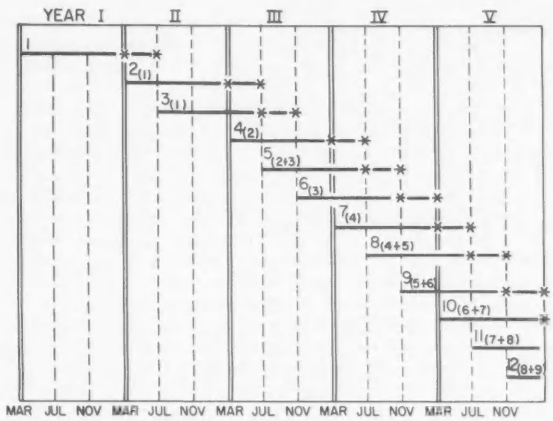

FIG. 4. Development of the population structure of *M. relicta* in Lake Michigan. Horizontal lines represent the life span of a cohort. Cohorts are numbered, with numbers in parentheses indicating the parent cohort. X indicates the release of young.

occur is from Reynolds and DeGraeve (1972). They presented evidence for migrations across depth contours at their shallow station. At their deep station, which was shallower than ours, they found a population reservoir where age-class distribution apparently did not change with time. Consequently, we believe our assumption to be correct.

The abundance estimates in this study compare favorably with estimates by other investigators at similar depths in Lakes Michigan, Huron, Superior, and Ontario. McWilliams (1970) reported mysid numbers ranging from 200 to 400 m^{-2} based on vertical net tows at 150 m in south-central Lake Michigan. Values in excess of 1000 m^{-2}, however, did occur in June and October of her April–November study. Reynolds and DeGraeve (1972), also working in southern Lake Michigan, but with an epibenthic sled, estimated abundances of 87 and 125 m^{-2} in January and April 1971 at 110 m. In Lakes Huron, Superior, and Ontario, Carpenter et al. (1974) calculated mean abundances of 97, 261, and 180 m^{-2}, respectively, based on vertical net tows at 100–125 m in spring and summer 1971.

The elucidation of the life cycle of *Mysis relicta* presented here could not have been accomplished without year-round sampling and a means of dividing the population into separate age categories. This life cycle, however, differs in many ways from earlier reports. Although all previous investigators have observed some reproduction in *Mysis* throughout the year: major reproductive activity was thought to occur only once during the year, usually in winter or spring (Juday and Birge 1927; Larkin 1948; Holmquist 1959; Lasenby and Langford 1972; Reynolds and DeGraeve 1972). McWilliams (1970) alone has concurred with findings in this study that juveniles are released at distinct intervals throughout the year. In her study at 150 m in Lake Michigan, she identified four such periods: late winter, mid-spring, late summer, and mid-fall. All but one of these periods generally coincide with major occurrences of juveniles presented in Fig. 2.

A small percentage (6% or less of mysids in any sample) of fourth instar females survived to molt to the fifth instar and breed again. These females, although small in number as compared to the total population, represent a significant portion of the breeding population. Since fifth instar females contribute significantly to the production of young, and since peaks in juveniles occur at intervals of 4 mo, fifth instar females are considered to release their second brood 4 mo after the first. In other words, a fourth instar female that released its first brood in March would molt to the fifth instar, mate, and release her second brood in July. It then appears that female *M. relicta* exhibit a 16-mo life cycle with release of young at 12 and 16 mo. The lack of fifth instar males suggests that males die soon after mating at 1 yr of age.

Several investigators (Larkin 1948; Holmquist 1959; McWilliams 1970) have suggested that female *M. relicta* can produce two broods, but the evidence in support of this has been largely circumstantial. The additional evidence provided by this study, that brooding females were observed in instars IV and V, is strong enough to make the possibility of two broods a near certainty.

Several investigations of the growth and life history of *Mysis* in temperate lakes have shown, in agreement with the present study, that females reach 14–16 mm in length and produce their first brood at the end of 1 yr. (Larkin 1948; Lasenby and Langford 1972; Reynolds and DeGraeve 1972). There seems to be little question or controversy about this first portion of the life cycle. It is concerning what happens next that the various studies fail to agree. The results of this investigation indicate that after the release of the first brood, females molted, attained lengths of 17–19 mm, mated, and released their second brood about 4 mo later. Larkin (1948) indicated that a second brood was produced by 18-mm females in Great Slave Lake, but he believed that this occurred a year after the release of the first brood. Lasenby and Langford (1972), however, showed females in Stoney Lake attained lengths of 18 mm approximately 4 mo after they had released their first brood, but did not find these females to be brooding young.

There are major discrepancies among these reports and the present one. The differences could be attributed to the fact that these are different populations in different localities. However, similarities to results in this study concerning the size of females that produced second broods in Larkin's study and the time it took females to reach this size in Lasenby and Langford's study are striking. The life history presented here may be widespread.

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Summer Phytoplankton Variability in the Lower St. Lawrence Estuary

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SINCLAIR, M. 1978. Summer phytoplankton variability in the lower St. Lawrence estuary. J. Fish. Res. Board Can. 35: 1171–1185.

The study involved two data series, a weekly sampling program at a single station near Rimouski from June through September, 1974, during which semidiurnal tidal effects were considered, and a 5-d survey of the lower St. Lawrence estuary during July 1975. The biomass and species composition distributions during the survey appeared to be a function of the residual circulation and mixing characteristics of the lower estuary. Active growth in phytoplankton populations was limited to a 2-mo midsummer period. The impoverishment up to late June was probably due to high turbidity, the 1% light level being about 4 m in depth, rather than due to advective flushing. Strong temporal fluctuations in water column phytoplankton biomass and production were similar to the distribution in density stratification. The confidence limits of the derived parameters [photosynthesis at light saturation normalized by chlorophyll (θ) and by carbon (μ_{pot}), and the carbon/chlorophyll ratio] were broad with respect to their seasonal range. Due to the lack of coherence in the physiological state indices considered, and the importance of species composition differences in "explaining" the observed distributions of the indices, the utility of the derived parameters as measures of physiological state of mixed populations is questioned.

Key words: phytoplankton, St. Lawrence estuary, primary production, physiological state, tidal mixing

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La présente étude porte sur deux séries de données : l'une recueillie lors d'un programme d'échantillonnages hebdomadaires à une station unique près de Rimouski entre juin et septembre inclusivement en 1974, au cours duquel nous avons étudié les effets d'une marée semi-diurne; l'autre recueillie lors d'un relevé de 5 jours dans le bas-estuaire du Saint-Laurent en juillet 1975. La répartition de la biomasse et de la composition par espèce lors du relevé semble être fonction de la circulation résiduaire et des caractéristiques des mélanges dans le bas-estuaire. Une croissance active des populations phytoplanctoniques ne se fait sentir que durant 2 mo à la mi-été. Le faible volume de phytoplancton observé jusqu'à la fin de juin est probablement dû à la forte turbidité (le niveau de 1% de luminosité se trouvant à environ 4 m de profondeur) plutôt qu'à la vidange advective. Les fortes variations temporelles de la biomasse et de la production phytoplanctoniques dans la colonne d'eau sont semblables à la distribution dans une stratification par densité. Les limites de confiance des paramètres dérivés [photosynthèse à saturation lumineuse normalisée par la chlorophylle (θ) et par le carbone (μ_{pot}), et le rapport carbone/chlorophylle] sont étendues par rapport à leur éventail saisonnier. Le manque de cohérence dans les indices de condition physiologique considérés, de même que les grandes différences qu'on observe dans la composition par espèce lorsqu'il s'agit d' « expliquer » la distribution des indices, nous font douter de l'utilité des paramètres dérivés comme mesures de la condition physiologique de populations mixtes.

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IN addition to generating in certain estuaries conditions which are favorable to production over long periods (Riley 1967) and in which temperature is an important parameter (Williams and Murdoch 1966; Mandelli et al. 1970), the dynamic nature of the estuarine circulation and mixing may result in highly variable distributions of phytoplankton biomass and production both spatially and temporally within the period of active growth (eg. Loftus et al. 1972; Winter et al. 1975). It is this variability that has perhaps hindered the development of a general understanding of estuarine phytoplankton dynamics since it causes extremely difficult logistical problems relative to sampling. If tides are of importance within the area of interest one might expect equal magnitudes of variability at several time

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scales (Welch and Isaac 1967). And if the sampling design does not incorporate "averaging," as has been standard procedure in physical studies, the interpretation of discrete samples taken irrespective of the various phases of the tide (eg. semidiurnal, neap-spring) is difficult.

A partial aim of this study is to delimit the seasonal cycle of phytoplankton growth and to describe the nature of the fluctuations within this period, within the lower St. Lawrence estuary, taking into consideration the importance of a sampling program which identifies tidal effects. It is hoped that an adequate description will suggest the causal mechanisms controlling the observed variability. In previous phytoplankton studies within this estuary the sampling frequency was at best biweekly and the observed distributions are difficult to interpret (Steven 1974).

In addition to measures of phytoplankton biomass, production, and species composition, estimates of the physiological state of the mixed population are considered. Platt and Subba Rao (1975) have reviewed the literature on possible indices and their applications in the field. And Eppley (1972) has suggested the predictability of maximum growth rate from temperature. In this study the assimilation ratio (θ, photosynthesis at saturating light intensity normalized by chlorophyll a), the cellular carbon to chlorophyll a ratio (C/chlor) and the potential growth rate (μ_{pot}, the growth rate measured under saturating light conditions) are critically evaluated as physiological indices. It was expected that these simply measured indices would aid in interpreting the temporal distribution in biomass and production.

Materials and Methods

The study involved a weekly sampling program at a single station near Rimouski from June through September during 1974, and a 5-day survey of the lower St. Lawrence estuary during July 1975. The stations occupied during these cruises are indicated in Fig. 1. During the summer of 1974, the station was occupied, usually each Monday, from 09:00 to 16:00. After the Secchi disk reading and a bathythermograph profile were taken, a six depth hydrocast (0, 2, 5, 10, 20, 30 m), was obtained using 12-L Niskin bottles equipped with reversing thermometers. ^{14}C innoculated subsamples taken from each Niskin bottle were replaced at their source depths. To minimize shading of surface samples, the bottles were strung from a plastic tube supported at each end by buoys.

Concurrently to arranging the in situ experiments, 12 subsamples were drawn from the Niskin bottle from 2 m depth, innoculated with ^{14}C and placed in three PVC transparent tubes (four light bottles in each tube) within a deck incubator. Each tube was maintained at a different temperature by pumping water from variable depths. The specific depths were chosen from the bathythermograph profile to include the full natural range of temperature. The resultant radiation in one plane within the deck incubator, measured with an Eppley Black and White pyranometer placed on the bottom of the incubator, was approximately 10 g cal $\cdot$ cm^{-2} $\cdot$ h^{-1} (1 joule/s = 14.34 g cal/min); which is sufficient for saturation of photosynthesis, but not high enough to cause photoinhibition. This assumption was tested empirically by photosynthesis versus light intensity curves estimated by using neutral density filters within the incubator. To check the assumption of a lack of nutrient limitation on phytoplankton growth for the region of sampling, each week two of the 12 bottles in the incubator were spiked with 5 mL of nutrient rich water, such that the final concentration was estimated as approximately 25 μg-at $\cdot$ L^{-1} of NO_3, 5 μg-at $\cdot$ L^{-1} of PO_4 and 25 μg-at $\cdot$ L^{-1} of silicate (in addition to the original nutrients in the sample). The ^{14}C uptake of the spiked samples was never outside the range of the untreated samples. The in situ incubation provided an estimate of primary production, whereas the deck incubation provided estimates of the average physiological state of the mixed populations as a function of temperature.

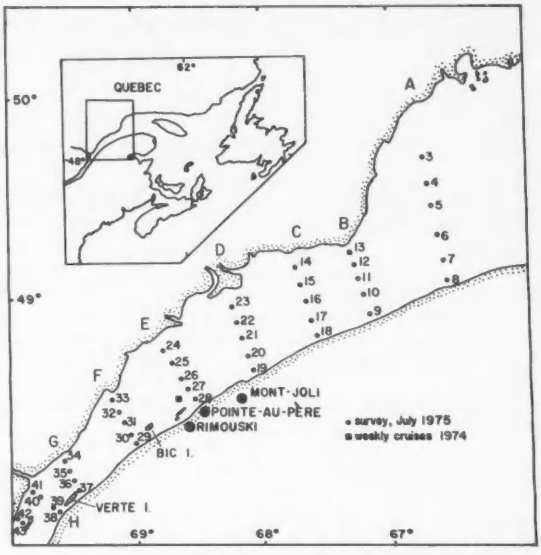

FIG. 1. Location of the stations sampled within the lower St. Lawrence estuary during this study.

The total time from the dropping of the messenger to the positioning of the BOD bottles, both in situ and in the deck incubator, was at most 30 min. In situ incubation lasted 6 h; deck incubation, 5 h. The temperature of cooling water from the three incubation tubes was measured every 15 min. After positioning the productivity bottles, samples (in triplicate for the biomass estimates) were drawn from each Niskin for estimation of particulate ATP (adenosine triphosphate), chlorophyll a, phytoplankton species composition, alkalinity, and salinity; and the reversing thermometers were read. Phytoplankton species were preserved with neutralized formalin (1%). The ATP and ^{14}C subsamples were prefiltered through 404-μm Nitex netting to remove larger zooplankton.

Secchi disk readings and Nansen bottle hydrocasts (for temperature, salinity, and chlorophyll) were repeated at the same depths at 12:00 and 15:00 h. Incident radiation during the 6 h was measured continuously by and Eppley Black and White pyranometer mounted at an elevated position on the ship. Daily radiation was measured at the University in Rimouski with a solar radiation recorder (Weather Measure

Corporation model R 401) and wind velocity and direction taken hourly at the Mont-Joli airport. Tidal height readings were obtained from the nearby Pointe-au-Père tidal station (Fig. 1).

During the survey of the lower estuary, at each station (Fig. 1), a water sample was taken at 5-m depth with a 30-L Niskin bottle. A portion of the sample was prefiltered with 15- and 202-μm mesh Nitex netting, respectively, to remove the larger zooplankton and also to divide the phytoplankton biomass into two size classes. Subsamples for the two size classes (<15 μm, <202 μm) were drawn for the estimation of ATP and chlorophyll *a*. In addition, nonprefiltered subsamples were taken for estimation of phytoplankton species composition.

Chlorophyll *a* was determined by the modified method of Yentsch and Menzel given in Strickland and Parsons (1972). The particulate ATP samples were assayed by the luciferin–luciferase method of Holm-Hansen and Booth as described in Strickland and Parsons (1972) and transformed to living carbon using a constant (250, Holm-Hansen 1970). Sakshaug (1977), although he reports an order of magnitude range in the carbon/ATP ratio, suggests that under nonnutrient limited conditions and where growth rates are near maximal the constant ratio of Holm-Hansen is appropriate. The assimilated ^{14}C was counted by liquid scintillation (Nuclear Chicago model). The efficiency of the system varied from between 60 to 80% and external standardization was used to correct for quenching. Nonbiological adsorption of ^{14}C to filters was evaluated by zero-time controls (Williams et al. 1972). Carbonate concentrations were calculated from the alkalinity and pH measures following the methods described in Strickland and Parsons (1972). The specific growth rate $\mu = \frac{1}{C}\frac{dc}{dt}$ was calculated from the primary production (from either the in situ incubation or the deck incubator) and ATP estimates, following McAllister et al. 1964, and has the units h^{-1}. Species composition was estimated using the Utermöhl technique (Lund et al.1958) with an inverted phase contrast microscope (Wild M40).

Results and Discussion

Temporal Distributions Observed During the Weekly Sampling Program (June–October 1974) at the Rimouski Station

The degree of vertical stratification in the physical properties of the water column varied in a surprisingly regular manner. The water column was alternately well mixed or relatively stratified. This marked biweekly behavior is well demonstrated by following the weekly sequence (B to T) of T–S‰ curves of a standard depth interval (0–30 m) (Fig. 2). The sequence of alternating stratification levels indicated by the relative length and slope of the curves breaks down during only a single 2-wk period (I,J). In Fig. 2, the T–S curves are calculated in most cases from the first of the three hydrocasts taken on each sampling day, at approximately 09:00 h, irrespective of the phase of the semidiurnal tide. However, it is clear from Fig. 3 that the observed weekly variability was not a function of sampling at variable phases of the semidiurnal tidal cycle. The weekly differences in the T–S‰ curves are of a different nature

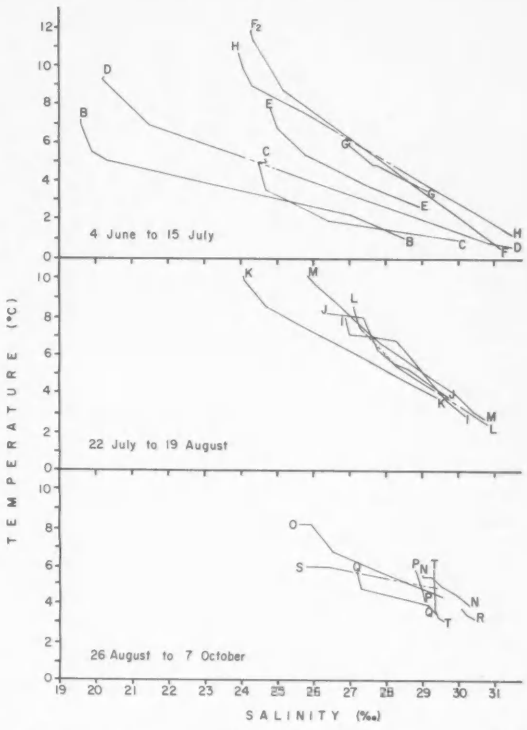

FIG. 2. T–S‰ curves observed at the Rimouski station during cruises B to T during the weekly sampling program, June–October 1974 (the dates for each cruise, B through T, are shown in Table 1).

(changes in slope and length) than the short-term variability observed over a tidal cycles (changes in length only).

Superimposed upon the above weekly variability is the expected seasonal variability in vertical stratification due to variable freshwater input into the estuary and the gradual change in day length. The radical nature of the temporal variability, both seasonal and weekly, in the vertical distribution of temperature, salinity, and density is clearly represented by the respective isolines (Fig. 4).

The temporal distribution in the degree of stratification of the water column (the difference in density between surface and bottom water), the daily radiation, the average daily wind speed and the maximum daily amplitude in tidal height are shown in Fig. 5. Although there is not a perfect match between the troughs and peaks of tidal amplitude and density stratification, which would be surprising considering the sampling frequency, there is an identical number of troughs and peaks. The wind and radiation curves, even though smoothed by 3-d running means, have more irregular distributions. Variable wind mixing might be expected

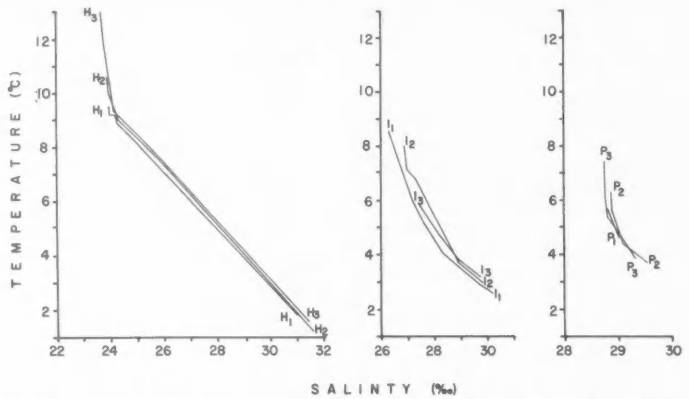

FIG. 3. Repetitive T–S‰ curves during a 6-h period on 3 sampling days; H, I, P.

to influence the level of stratification of the upper few metres. However, the observations shown in Fig. 4, in which the salinity and temperature characteristics of the whole water column varied from week to week, suggest that wind effects on the water column stratification are not sufficient to explain the observed distribution. The observed regular variability in the physical structure can be plausibly interpreted as due to variable tidal mixing intensity following the "neap-spring" cycle (M_f).

It is of interest that the turbidity of the surface waters, as estimated by Secchi disk readings, has a temporal distribution similar to the density stratification (Fig. 5). The surface water was relatively more turbid during stratified weeks. The high coefficients of extinction observed relative to the range in phytoplankton biomass observed (Fig. 6), suggest that the variability in turbidity is not principally a function of the phytoplankton biomass fluctuations. The distribution suggests, however, that the suspended load (of presumably nonliving sediment) is higher in the low salinity surface waters. When this surface water is mixed during periods of increased tidal intensity, the suspended load may be distributed through the water column more evenly and result in a reduction of surface water turbidity. The sharp decrease in turbidity of surface waters at the end of June was probably due to the decreased freshwater input into the estuary and was of importance to the phytoplankton production (Fig. 6).

Depth integrated phytoplankton biomass and production are estimated from the chlorophyll and ATP profiles observed during the first hydrocast of each sampling day and the subsequent in situ 6-h incubations. Since replicate subsamples were taken for each of the measures, variances can be calculated for the integrated estimates (Platt and Irwin 1968). The average coefficients of variation ($100\, S_{\bar{x}}/\bar{x}$) for the three integrals are indicated on the bottom line of Table 1. The precision in the ^{14}C method (coefficient of variation as high as 28%) is very much less than the precision for the biomass estimate; the result is that confidence limits for the water column primary production are broad, approximately ±50% of each estimate. Daily primary production was estimated following the method of Platt (1971).

The weekly distribution of the water column estimate of chlorophyll *a*, daily primary production and $\nabla\sigma_t$ are plotted in Fig. 6. There are two points of interest. The temporal fluctuations in biomass and production are similar to the distribution of water column stability ($\nabla\sigma_t$), except perhaps during September. Also, the water is relatively impoverished during early June in spite of the marked stratification in density and the high daily radiation level. From inspection of Fig. 7, in which the vertical distribution of biomass and primary production are presented together with the depth of the 1% light level, it appears probable that the high turbidity of the surface waters during the first 3 wk of June may have contributed to the low production level. During this period of high turbidity, the 1% radiation depth is about 4 m in depth. The importance of the physical structure, which is thought to be predominantly a function of variable tidal mixing, on the phytoplankton biomass and production distribution is clear from Fig. 4 and 7.

The repeated hydrocasts (09:00, 12:00, 15:00 h) at the same station on each sampling day permit the evaluation of the importance of tidal variability of a semidiurnal nature on the biomass estimates (chlorophyll only) in relation to the variability observed between weeks. It was indicated, during the description of the T–S curves, that the semidiurnal tidal variability (that observed over a 6-h period in this study) is considerable but it is not of sufficient magnitude to mask the frequently radical differences in the S‰ and temperature profiles observed between weeks. In Fig. 8, the short-term (hourly) variability in surface temperature and chlorophyll *a* is indicated in relation to both the

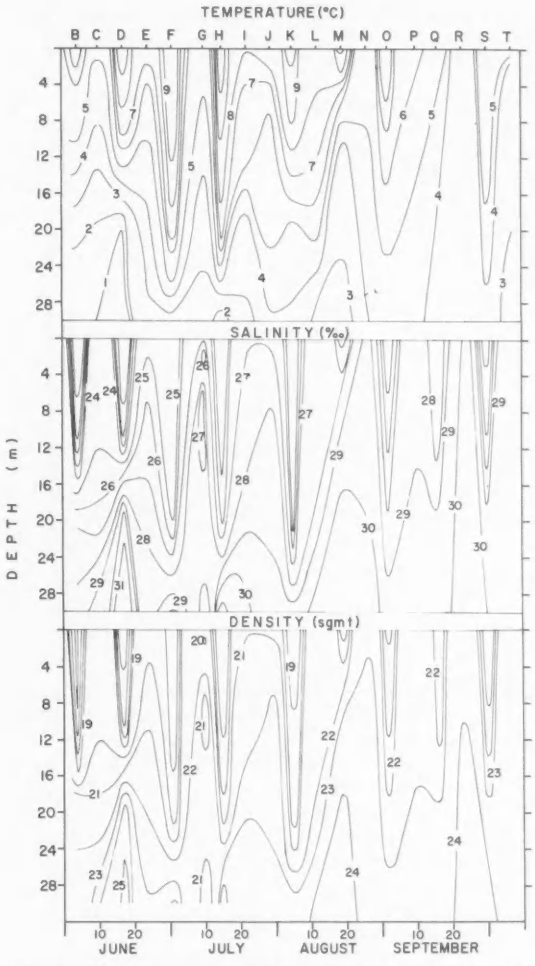

FIG. 4. Temperature, salinity, and density isolines observed at the Rimouski station during the weekly sampling program, June–October 1974.

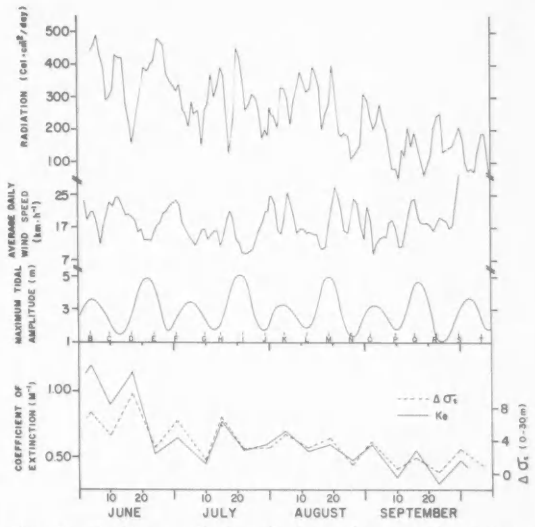

FIG. 5. Incident radiation (3-d running mean), average daily wind speed (3-d running mean), and maximum daily tidal amplitude observed in the Rimouski area during the weekly sampling program, June–October 1974. The weekly distribution in density stratification and surface-water turbidity (Ke represents coefficient of extinction) observed at the Rimouski station during the same period are shown in the lower section (1 joule/s = 14.34 cal/min).

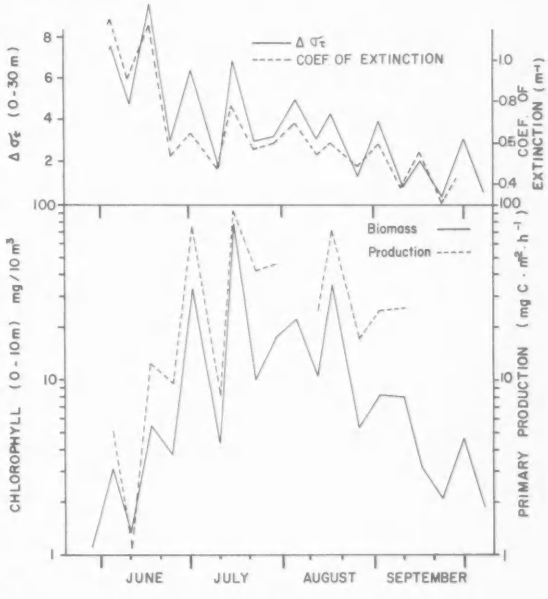

FIG. 6. The temporal distribution of surface layer biomass and primary production, as well as water column density stratification and surface layer turbidity, that were observed at the Rimouski station during the weekly sampling program, June–October 1974.

weekly variability and the standard deviation associated with the analytical error (for chlorophyll *a*). The estimated semidiurnal variability in surface layer chlorophyll *a*, even though of considerable magnitude, is relatively small in comparison with the observed week-to-week variability. Thus, the observed weekly variability in biomass, and by inference production, is not a function of sampling at different phases of the semidiurnal tide. There are "events" of sufficient magnitude occurring over a period of days that they are observable in spite of the large semidiurnal tidal component of variability.

The phytoplankton species composition observed during the above sampling program is shown in Table 2. Although there is not a gradual succession in species

TABLE 1. Water column estimates (with standard errors) of phytoplankton biomass and production at the Rimouski station (June–September 1974).

Cruise	Date	Biomass: Chlorophyll *a* (mg/m^2)	Biomass: Carbon (mg/m^2)	Primary production ($mg\ C \cdot m^{-2} \cdot h^{-1}$)	Incubation time (h)	*F* value	Daily primary production ($mg\ C \cdot m^{-2} \cdot day^{-1}$)
B	4.06.74	5.48±0.45	165.2±9.8	5.2±0.73	6.4	0.72	46.1±6.44
C	10.06.74	2.28±0.31	306.9±21.6	1.1±0.15	6.1	0.71	9.5±1.30
D	17.06.74	9.43±0.49	585.0±21.1	12.3±2.02	6.0	0.68	108.5±17.84
E	24.06.74	7.74±0.28	556.1±34.9	9.6±2.24	6.0	0.71	81.2±18.98
F	1.07.74	50.10±2.80	6024.6±401.4	74.0±6.73	6.2	0.71	660.9±60.10
G	10.07.74	7.42±0.24	498.6±12.1	7.9±2.25	6.0	0.69	68.9±19.61
H	15.07.74	229.86±5.14	5771.7±570.8	92.8±17.56	6.0	0.69	802.1±151.75
I	22.07.74	32.87±1.13	894.5±73.2	42.9±5.65	6.0	0.70	368.9±48.57
J	29.07.74	34.43±2.13	1407.5±149.3	45.9±12.70	6.0	0.71	390.6±108.06
K	5.08.74	51.47±3.87	1167.4±110.4				
L	12.08.74	28.81±1.97	1285.5±77.9	24.7±3.64	6.0	0.72	207.1±30.50
M	19.08.74	50.02±2.68	1716.7±69.9	70.7±14.24	6.0	0.74	575.1±115.85
N	26.08.74	12.97±0.85	931.9±39.0	17.5±1.24	6.0	0.75	139.6±9.93
O	2.09.74	27.58±0.61	993.4±22.4	25.4±4.47	6.0	0.76	198.9±34.98
P	10.09.74	23.94±0.71	1236.0±67.4	26.3±6.74	6.0	0.78	201.8±51.75
Q	16.09.74	10.26±0.20	399.0±30.3	3.5±0.78	3.3	0.48	23.4±5.20
R	23.09.74	7.42±0.20	479.7±56.7	17.0±2.99	6.0	0.79	128.4±22.61
S	1.10.74	7.70±0.44	802.2±44.7				
	% C.V.	5.1±2.8	6.5±2.7	18.1±6.2			
	Range	1.9→13.6	2.3→11.8	7.1→28.5			

composition through the summer (e.g. week F, 6 new species; week H, 4 new species; week I, *Melosira* species occurs abundantly), the species, after their sudden appearance in high concentration in the water, were in many cases present for several weeks. The dominants that are abundant during a "stratified" high biomass week were frequently also in the water during the following or previous less stratified, relatively low biomass week (e.g. *Skeletonema costatum* and *Nitzschia* species are dominants during weeks B, C, D; *Thalassiosira* species 2, 3 and *Melosira* species are abundant during weeks I, J, K). This supports interpreting the alternating physical conditions as being partially a function of variable intensity of tidal mixing of a discrete water mass, at least over a 2–3-wk period, rather than regular advection of two water masses back and forth past the Rimouski station. In the latter condition one would expect a radical shift in the species composition from week to week, with perhaps alternate weeks being relatively similar if species succession is slow. Clearly the sudden appearance of species of high abundance suggests the advection of new parcels of water past the station was occurring. The point made here is that there is not a regularity in species composition changes following the regular variability observed in the S‰ and temperature characteristics of the water column (Fig. 4). A second point is that frequently the phytoplankton biomass was composed of several species of comparable abundance. Thus, chlorophyll and primary production estimates did not in general originate only from a few dominant species. This becomes important in the interpretation of the physiological state measures on the mixed population.

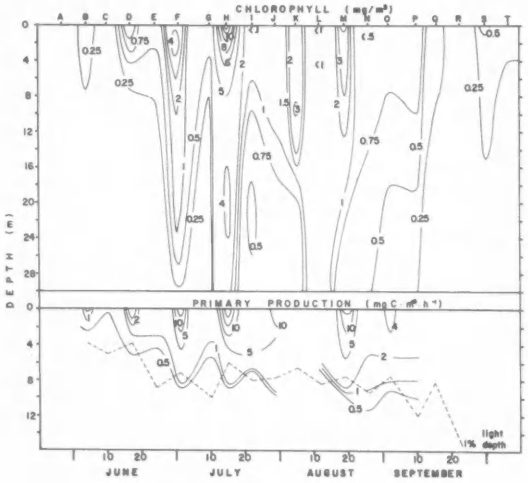

FIG. 7. Chlorophyll *a* and primary production isolines observed at the Rimouski station during the weekly sampling program, June–October 1974.

It is against the background of the above data on the distributions in physical characteristics of the water column, biomass, production, and species composition that the variability in physiological state is considered. Because the sampling design systematically involved

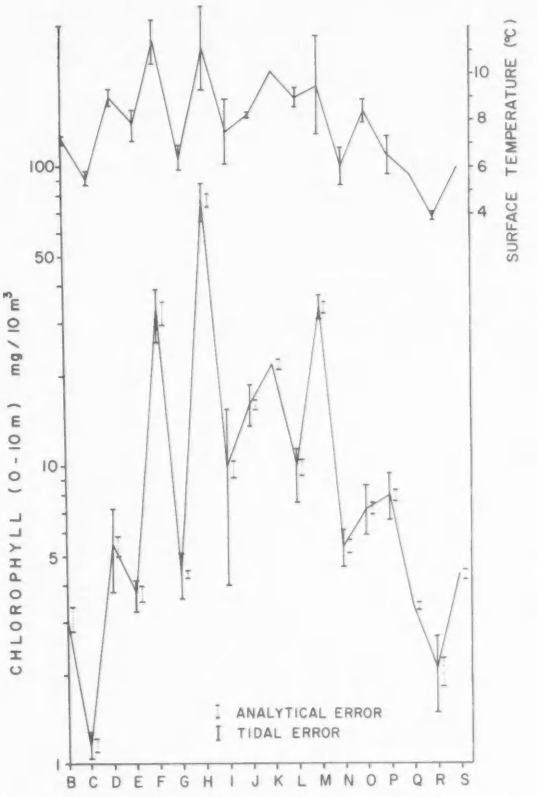

FIG. 8. Comparison of the weekly variability in surface temperature and surface layer chlorophyll concentration with the tidal and analytical error. The error bars are the standard errors.

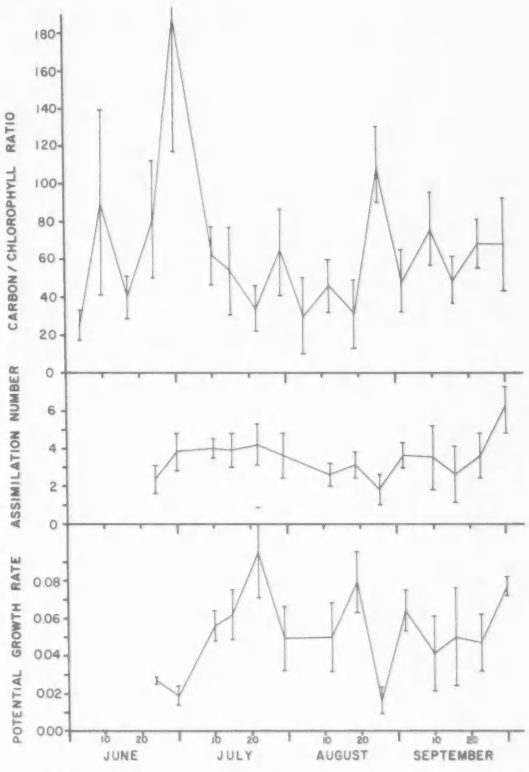

FIG. 9. The derived parameters (θ, μ_{pot}, C/chlor ratio) observed at the Rimouski station during the weekly sampling program, June–October 1974. The vertical error bars indicate the 95% confidence limits.

replicate subsamples for the source data (chlorophyll, ATP, ^{14}C) used in the estimation of potential growth rate (μ_{pot}), assimilation (θ), and C/chlor ratio, standard errors can be calculated for the estimates (following Platt and Filion 1973). In Fig. 9, the physiological state estimates for each sampling day are plotted with 95% confidence limits. The confidence limits are broad with respect to the seasonal range of the three parameters; hence, their usefulness as a physiological index is questionable. This is particularly critical for θ, for which most of the values over the 4 mo of sampling, during a broad range of environmental conditions, lie between 3 and 5. μ_{pot} (h^{-1}) and the C/chlor ratio show considerably more seasonal variability with respect to the confidence limits, but are subject to a possible lack of accuracy (nonphytoplankton ATP in the samples will tend to underestimate the growth rate and increase the C/chlor ratio, and lead to an apparent inverse relationship between these variables).

With the above limitations in mind (i.e. the broad confidence limits and the questionable accuracy of μ_{pot}) the seasonal distribution of μ_{pot} and θ, estimated at surface temperature, are plotted in conjunction with water column chlorophyll, primary production, and surface temperature (Fig. 10). μ_{pot} and θ do not behave similarly, and neither parameter reflects the fluctuations in biomass or temperature.

In spite of the lack of a temperature effect in the field, there is a marked effect of the experimental temperature during the shipboard incubations on μ_{pot} and θ (Fig. 11). The general effect of temperature on μ_{pot} and θ is evident even though there is lack of precision in the individual estimates (the 95% confidence limits for the high temperature estimates from each cruise are shown in Fig. 9). The most striking feature is the separation of the μ_{pot} curves relative to the clumping of the θ curves. This difference in seasonal range between the two measures was indicated above (Fig. 10) but is more emphatically presented in Fig. 11. The μ_{pot} curves M and I, which are higher than the rest of the curves, are associated with low C/chlor ratios, 34 and 31, respectively; the low μ_{pot} curves, F and N are associated with higher than average C/chlor ratios,

TABLE 2. Species composition and abundance (cells/litre) observed at the Rimouski station (2 m depth) during the weekly cruises (Summer 1974).

Centric	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
Asterionella formosa								2600			7600	20000						
Asterionella sp.																		
Chaetoceros debilis												46500	120	1400	1500	180		150
Chaetoceros difficilis							8800	2000	12500									
Chaetoceros septentrionalis										3000								
Chaetoceros socialis										82000								
Chaetoceros sp.									900	2000								
Coscinodiscus 1	90	60	120															
Coscinodiscus 2						60				500								
Eucampia groenlandica									1500									
Fragilaria sp.	80	60																
Melosina sp.								176800	91500	30500		62500						
Skeletonema costatum	4500	1340	840															
Stephanopyxis														150				
Thalassiosira 1							4320		260	220	340			20	50			200
2							10000	13800	12500	41000		14500	1500	2400	1650	380	480	610
3							8200	14000	11000	25500		28000	4000	120				
4											1600							
Thalassiothrix nitzschoides	10									7500	80							
Thalassiothrix sp.																		
Pennate																		
Amphora sp.				20							400							
Grammatophora								800										
Licmophora			80	120													400	
Naviculoids	280	390	960	441	931	60	1800	5800	1500		1200	1500			200	1000	2400	800
Nitzschia closterium				180														
longissima	100	210	1160	60			40			500								
delicatissima							8000	8400	41500									
seriata							980		100	100								
Phaeodactylum tricornutum	160	50	560	16560	1596	120	10^6	5000	171000	9000	23600							
Dinoflagellates																		
Ceratium sp. 1 (tripos)					60					40								
Ceratium sp. 2													200	1650				
Dinophysis sp. 1					1596		200								100			
Gonyaulax tamarensis					57456	80	33200				3600			250	1000	40		
Gymnodinium sp.						400					320		30	30	30	180		
Minusculium bipes					133													
Peridinium 1					1596		19800			10000								
Peridinium 2											140							
Peridinium oblongum (~100 μm)					160													
Peridinopsis sp.						20												
Others																		
Chlorophyte (Scenedesmus type)	10									500	400		500	50				400
Flagellates															900	700	800	4800
Tintinnids	20			120	20		2120		4540	320	420	200	70	260	240	700	620	450
Nauplius larvae 1																		10

188 and 110, respectively. The low μ_{pot} and the high C/chlor ratios during weeks F and N may be due to the presence of *Gonyaulax tamarensis* and *Ceratium* sp. These four periods (M, I, F, N) are clustered together when production is normalized by chlorophyll (θ). One would not expect the overall distribution shown in Fig. 11 to be effected by "contamination" of the ATP samples unless certain weeks have much higher microzooplankton/phytoplankton biomass ratios. If "contamination" is a significant problem one would doubt the low μ_{pot} and high C/chlor ratios. From Table 2, the abundance of tintinids, essentially the only microzooplankters observed during the phytoplankton species counts, during weeks F and N does not suggest that the low μ_{pot} values are suspect. The more probable effect of microzooplankton "contamination" is to raise the variance of the ATP estimates due to occasional inclusion of a high proportion of animal ATP in the 150-mL subsample. Since each of the μ_{pot} estimates in Fig. 11 is derived from triplicate ATP subsamples, the effect of contamination by microzooplankton on the overall distribution should be slight. However, variation in the bacterial biomass could be of importance.

An equation relating maximum expected growth rate to temperature (Eppley 1972) has been transformed such that μ_{max} is expressed in the units hour^{-1} (log to the base e) rather than in doublings per day and is plotted over the temperature range of interest. A large portion of the μ_{pot} estimates fall above the suggested maximum. This is thought to be due to the difference in methodology used to estimate growth rate in this study relative to that used in the data reviewed by Eppley. Eppley's curve was derived from a review of the literature on phytoplankton growth rates of individual species under batch culture conditions. In such studies, growth rate is calculated from cell count differences over 24-h periods. In this study, μ_{pot} is estimated from ^{14}C uptake during a 5-h incubation. McAllister et al. (1964) using the latter methodology, but with individual species, also report values considerably above the μ_{max} curve. The high μ_{pot} estimates from the 5-h incubations, in comparison to the 24-h estimates

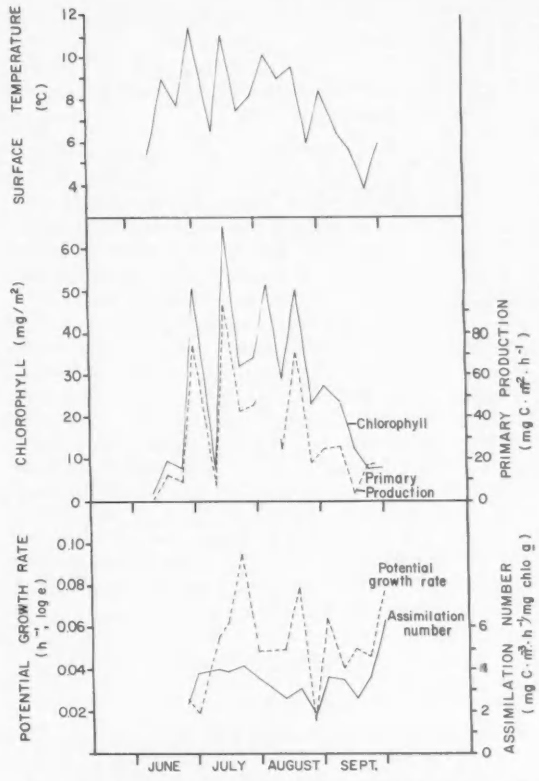

FIG. 10. Comparison of the temporal distribution of the derived parameters observed at the Rimouski station during June–October 1974, with that of surface temperature, chlorophyll concentration, and primary production.

in the literature, may be a result of incubating the samples during the active portion of the diel cycle of photosynthesis.

The in situ growth rate and production per unit chlorophyll are plotted with respect to the photosynthetically available radiation (P.A.R.) at each depth (Fig. 12). The two sets of curves have a different form which is evident in spite of the small number of data points for each curve (3 or 4). The production per unit chlorophyll versus P.A.R. curves are mostly asymptotic whereas the growth rate versus P.A.R. curves often peaked at intermediate values of P.A.R. The different shape is a function of the general decrease in C/chlor ratio with depth (Fig. 13). The surface samples, which have a relatively high C/chlor ratio, have a lower growth rate than the 2-m samples; the production per unit chlorophyll is, in general, essentially the same for both depths. This suggests that the increased C/chlor ratio in the surface samples is not a classical case of sun adaptation. If this were so, one would expect a higher production per unit chlorophyll in the surface samples of higher illumination (Strickland 1965). The distribution of growth rate, with frequently lower growth rates at the surface than estimated at 2 m depth, suggest rather that the elevated C/chlor ratios are an indication of reduced physiological vigor, or that the increased ratio is characteristic of highly illuminated populations that provides no physiological advantage and as such, is not truly an adaptation. Irrespective of whether the high ratios are an indication of "sun adaptation" or of physiological stress, they appear to be a function of the higher illumination of surface waters. However, differential species composition with depth, with a higher proportion of slow-growing dinoflagellates close to the surface, could cause the same effect. There are two clear exceptions: cruises

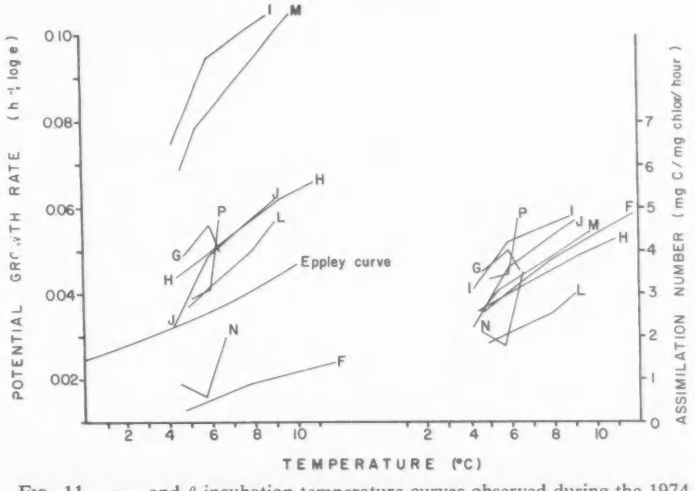

FIG. 11. μ_{pot} and θ incubation temperature curves observed during the 1974 weekly sampling program at the Rimouski station.

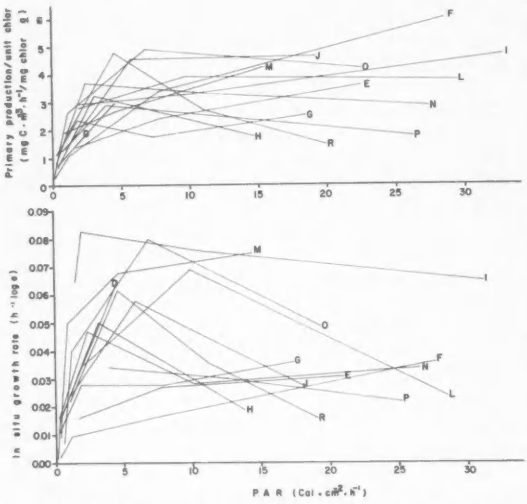

FIG. 12. Chlorophyll normalized in situ primary production and in situ growth rate versus P.A.R. curves observed during the 1974 weekly sampling program at the Rimouski station.

D and G, where the C/chlor ratio increases with depth (Fig. 13). The increasing ratios with depth may represent phytoplankton populations that are out of equilibrium with their present light regime. The displaced μ_{pot} curves for weeks F and N (Fig. 11) are associated with unusual C/chlor depth profiles (Fig. 13). During these 2 sampling days, dinoflagellates — which can maintain their vertical distribution in relation to a preferred light intensity — were dominant. The C/chlor distribution suggests that the dinoflagellate populations, which can have high cellular C/chlor ratios (Eppley and Sloan 1965), were aggregated at or close to 2 m from the surface.

SPATIAL DISTRIBUTIONS OBSERVED WITHIN THE LOWER ESTUARY (JULY 17–21, 1975)

The spatial distributions of chlorophyll and ATP concentrations at 5 m depth are shown in Fig. 14. The dominant feature is the higher biomass levels along the south shore. In spite of this cross channel structure, the species distribution observed was relatively similar along the north–south transects but different in the east–west direction (Table 3). For example, transects G and F were characterized by an unidentified but unique and visually observable detritus (large orange-brown flakes), low biomass, and no dinoflagellates and centric diatoms. In contrast, transects E and D are characterized by less detritus, higher biomass, and a different species composition (consisting of centric diatoms and dinoflagellate species). Again, this suggests that the temporal distributions observed in front of Rimouski are not a function of different types of water being advected past the station in a west–east direction, but rather are representative of a temporal sequence within a water mass within the lower estuary which appears to be maintained by a clockwise gyre.

General Discussion

DISTRIBUTIONAL ASPECTS

The phytoplankton biomass and species distributions observed within the lower St. Lawrence estuary parallel the major physical features. During the period of spatial sampling, the western end of the lower estuary, from the Verte Island to about Bic Island, was characterized by low biomass of restricted species composition (naviculoids and flagellates) and a unique form

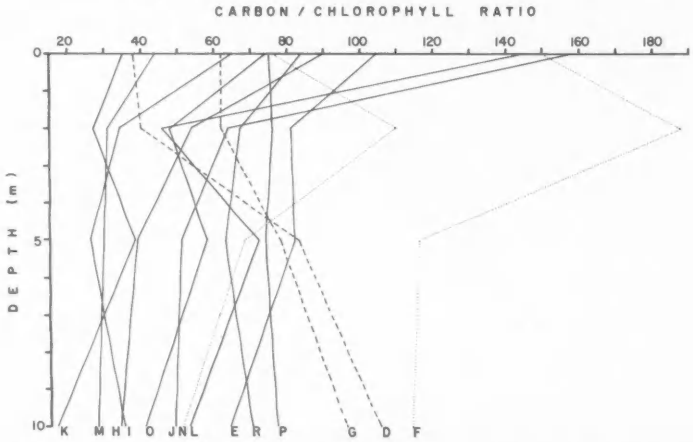

FIG. 13. C/chlor ratio depth distribution observed at the Rimouski station during the 1974 weekly sampling program.

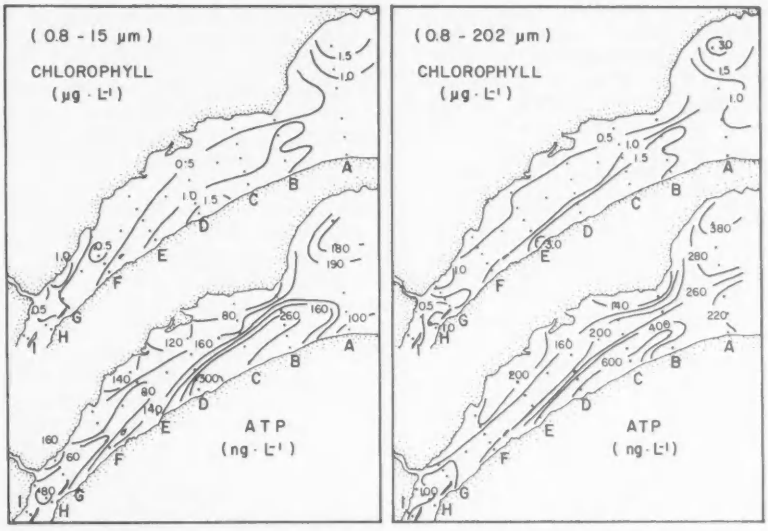

FIG. 14. Spatial distribution of chlorophyll *a* and ATP at 5 m depth during July 1975, within the lower St. Lawrence estuary.

of detritus of unknown origin (large orange flakes, 100–200 µm in cross-section). Several authors have described the exceptionally high amplitude internal tides within this region (e.g. Forrester 1974); and P. Greisman and R. Ingram (unpublished data) have suggested intense horizontal and vertical mixing due to the shear associated with interaction between the Saguenay–upper estuary outflow and the large amplitude vertical displacements. Such intense mixing and the associated high turbidity are not conducive to phytoplankton growth and may explain the low biomass in this region. The naviculoids may have been benthic forms scoured up into the water column.

TABLE 3. Relative species composition of phytoplankton within the lower St. Lawrence Estuary.

	Stations							
	29	31	33	28	26	24	19	21
Centric diatoms								
Chaetoceros sp.							2	
Melosira sp.				39			<1	
Thalassiosira 1				15				
Thalassiosira 2				20	55	78	1	14
Thalassiothrix sp.								11
Pennate diatoms								
Naviculoids	25	42	48		2	3		3
Nitzschia longissima					2		6	3
Dinoflagellates								
Dinophysis sp.					2			
Gonyaulax tamarensis				11	5	5	51	8
Gymnodinium sp.				2	7	3		27
Minuscula bipes						4		1
Peridinium ovatum							4	1
Peridinium sp.					5	3	10	19
Others								
Flagellates	19	28	25	6	9		22	13
Tintinnids	56	30	27	7	12	4	4	

The central part of the lower estuary, from Bic Island to at least section F, was characterized by a radically different species composition (Table 3) and considerably less detritus. In addition, there was a strong cross-channel gradient in biomass, with higher levels along the south shore (Fig. 14). This gradient parallels the salinity and temperature gradient of the surface layer; warmer and less saline water occurs along the southern shore. Neu (1970) attributes this salinity and temperature distribution to the cross-channel component of the residual downstream surface current due to the Coriolis effect and also suggests that there is a compensatory slow upwelling along the north shore. Such a circulation would favor the maintenance of a higher level of biomass along the south shore and account for the clearer, low-biomass water towards the opposite shore. In addition, the suggested residual surface-water clockwise gyre within this region (El-Sabh 1977), with the increased residence time of surface water, would tend to reduce the advective losses from the region thus permitting the higher biomass levels observed and the maintenance of a relative homogeneity in species composition.

With the stratification–circulation classification of estuaries (Hansen and Rattray 1966), the relative importance of tidal mixing and the intensity of the gravitational circulation in the two regions (between

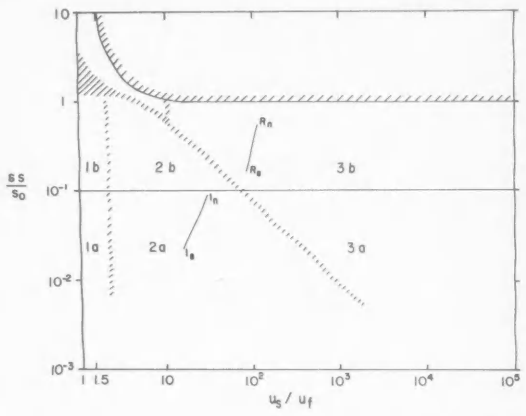

FIG. 15. Position of the Isle Verte and Rimouski sections within the stratification–circulation classification. I_s, I_n and R_s, R_n indicate spring and neap tide positions of the Isle Verte (I) and the Rimouski (R) sections.

sections G and E, and between sections E and B (Fig. 1)) can be inferred (Fig. 15). The Rimouski section, with greater stratification and vertical advection (as indicated by u_s/u_f), is a potentially more productive environment for phytoplankton than is the Isle Verte section. Also, within any one section of the estuary, the M_f tidal cycle has a strong effect on the character of the estuarine mixing and circulation and, thus, on the phytoplankton environment.

Certain features of the temporal variability in biomass and production observed at the Rimouski station during the 1974 sampling program (June–October) were also observed in Puget Sound by Winter et al. (1975). In both estuaries there is a short growing season, June to September for the lower St. Lawrence (Fig. 7), which is initiated by a late "spring bloom" for the respective latitudes. In addition, strong fluctuations were noted in biomass and production as well as density stratification over periods of days in Puget Sound, and from week to week in the lower St. Lawrence. The exceptionally late spring bloom in Puget Sound was not thought to be explainable in terms of Sverdrup's critical depth model (incident radiation and turbidity in relation to the depth of mixing). The importance of reduced residence time of surface water within the Sound due to large horizontal advection during high runoff periods was suggested as a likely explanation for the late bloom.

The growth period in the estuary follows the distribution in estimated critical depth, with deepening of the critical depth at week E, prior to the large increase in biomass (week F) and a decrease in critical depth at week O which coincides with the decrease in biomass (see Table 1). The factor that influences the critical depth value prior to the "bloom" is the rapid decrease in turbidity of surface water at the end of June. It is the shortening day length which is critical to the shoaling of the critical depth in September. In addition, frequent shallow critical depth estimates between June and September indicate the marginal character of this environment for phytoplankton growth.

The importance of turbidity in the initiation of summer phytoplankton growth at the Rimouski station is recognizable in Fig. 7, and, thus, the results of the critical depth calculations are perhaps not unexpected. The calculations are interesting nevertheless in that they indicate, because of the "goodness of fit," that within this estuary, horizontal advection during spring is probably not extremely critical.

Within the period of growth, essentially July and August for the lower St. Lawrence estuary, there are strong fluctuations in biomass and production from week to week. It was noted in Fig. 6, that the fluctuations parallel the distribution of surface-water stratification, and that the regularity of these fluctuations suggest that the distributions are controlled by the cyclic variability in the magnitude of tidal mixing following the neap-spring cycle. Clearly, the sampling frequency (once per week) is inadequate to document such a relationship appropriately (Fig. 5). However, the similar relationship observed by Winter et al. (1975) with almost daily sampling, strengthens the above interpretation. They note a partial positive correlation between tidal amplitude and density stratification, but do not discuss or point out a parallel distribution in biomass and production. From inspection of their figures, it appears that there was a strong relationship between primary production and density stratification during both 1966 and 1967 (Fig. 5 and 6 of Winter et al. 1975). In the former year, the parallel behavior does not begin until after mid-May. Prior to this period, there is a very anomalous tidal height distribution, and density stratification does not parallel either tidal amplitude, production, or biomass. To check these observations statistically, the paired values for stratification and primary production were read off their figures and correlation coefficients calculated. The data series are significantly correlated at the 1% probability level (1966, $N = 20$, $r = 0.55$; 1967, $N = 26$, $r = 0.82$). It is of interest that during the higher frequency sampling program, nearly daily during 1967, the correlation is strongest. Also biomass itself is, at least on inspection, not as well correlated with density stratification. This latter point is of interest, since it suggests that the processes influencing the variable density stratification have a strong impact on the production processes themselves, the dynamic aspects of phytoplankton growth, rather than being just a physical aggregation effect.

Clearly, the temporal fluctuations in surface-water density stratification in estuaries are a complex function of variable tidal mixing, freshwater runoff, and wind mixing. In deep estuaries, such as the lower St. Lawrence and Puget Sound, the latter effect may not mask the regularity of the tidal mixing effect (following neap-spring cycle). Thus, within periods of relatively constant freshwater runoff, the variable intensity of tidal mixing may have an important effect on stratification and

TABLE 4. Field variance in the derived parameters (% coefficient of variability).

	θ	μ_{pot}	C/chlor *a*
Rimouski station			
1974, June–October	29.1 (15)[d]	44.9 (15)	57.2 (17)
1975, 3½ days[a]	18.8 (36)	31.9 (36)	34.0 (36)
Chesapeake Bay[b]			
~20 km transect	19.0 (5)		
Puget Sound[c]			
April–June 1966	25.0 (21)		
Methodological error (from Sinclair et al. 1978)	12.8	11.4	13.7

[a]Calculated from Sinclair (1977) where stations 21 to 27 are omitted.

[b]Calculated from table 3 of McCarthy et al. (1974) using the assimilation numbers from samples at 1–2 m depth (prescreened, <163 μm).

[c]Calculated from fig. 8 of Winter et al. (1975).

[d]Brackets indicate number of samples.

phytoplankton growth. Increasing tidal mixing, with subsequent reduction in stratification, influences the phytoplankton population by increasing the rate of loss of cells from the euphotic zone due to the higher vertical diffusivity and decreased upward vertical advection. These effects are well illustrated by the position of the Rimouski station in the stratification–circulation classification during consecutive weeks (stratified–unstratified) in Fig. 15. For the above environments reduced stratification parallels a reduced primary production. In certain shallow estuaries, however, where the loss rate from the euphotic zone is less critical and nutrient limitation of the surface layer is an important consideration, a negative relation has been observed between stratification and primary production (Iverson et al. 1974; Parsley and Barber 1976). The increased nutrient concentration in surface water during reduced stratification is thought to stimulate production.

DERIVED PARAMETERS

The interpretation of the distribution of the derived parameters is critically dependent on confidence in the accuracy and precision of the measured parameters. ATP has been used as a measure of living phytoplankton carbon and thus caution is required in the interpretation of the derived parameters (μ_{pot} and C/chlor ratio). Since heterotrophic contamination will tend to underestimate growth rates and overestimate C/chlor ratios, at a minimum the major high and low values in the respective distributions should reflect real events.

A second methodological consideration is the precision associated with the biomass and production methods and, thus, the sensitivity of the derived parameters. The error associated with the methodology is discussed in Sinclair (1977), and this error is considered here in relation to the observed normalized field variance in the derived parameters over various space and/or time scales (Table 4). In this context, the limitations of the method in detecting spatial or temporal differences in the derived parameters is clear. The field variance of μ_{pot} and C/chlor ratio is considerably larger than that of θ, whereas the methodological errors for the three derived parameters are relatively constant. Thus, μ_{pot} and C/chlor ratio are more sensitive indicators of in situ heterogeneity whereas the relative constancy of assimilation ratio indicates the utility of chlorophyll as a measure of productive potential of a sample in the lower St. Lawrence estuary. The relative constancy of assimilation ratio in estuaries both spatially within Chesapeake Bay and temporally within Puget Sound is also indicated (McCarthy et al. 1974; Winter et al. 1975).

A final methodological problem is the physiological interpretation of the derived parameters. The measured parameters (chlorophyll, ATP, and primary production) are made on a mixed population with a certain species diversity. It is important to ask whether there is any physiological meaning in ratios of these parameters. The critical requirements for a meaningful field value are coherence between species in their physiological condition or marked dominance by one or two species over the period of sampling. These conditions are difficult to check in the field but if the various indicators of physiological state behave in a similar fashion in a field sampling program, one would have a certain confidence that the field methodology was actually measuring meaningful physiological responses of the mixed phytoplankton to environmental conditions. This was shown to be the case over a short time period by Platt and Subba Rao (1970). Correlations between several of the suggested indices estimated in this study are shown in Table 5. The chlorophyll *c* data, necessary for the calculation of the chlor *c*/*a* ratio, was provided by D. Lefebvre (unpublished data). The general lack of coherence between the "physiological" measures weakens the argument for their meaningfulness in field sampling. This point is particularly critical for the chlorophyll *c*/*a* values (observed range 1.36 to 2.39). In culture studies, no cellular values have been found greater than one (Jeffrey 1974). However, due to the stability of nonliving chlorophyll *c* relative to chlorophyll *a*, high values can be found in field samples due to the detrital chlorophyll *c* contribution to the ratio.

There is, however, the suggestion that the derived parameters estimated in this study are a useful tool in field sampling programs, in that changes in species composition are identifiable. High C/chlor ratios, low μ_{pot} values and intermediate assimilation ratios were associated with dinoflagellates (*Gonyaulax* sp. and *Ceratium* sp.) during cruises F and N. It is worth noting that the importance of these two species in the water column would not have been suspected if only θ was estimated. Mandelli et al. (1970) did note a lower assimilation ratio during dinoflagellate dominance but their presence is much more forcefully indicated with μ_{pot} and C/chlor ratio (Fig. 11). The low growth rates are perhaps ex-

TABLE 5. Correlation coefficients among the "physiological state indices" derived from the Rimouski station data collected during the summer of 1974. Each coefficient is based on 15 df. (*Exceeds value expected at the 5% probability level; **Exceeds value expected at the 1% probability level.)

	θ	μ_{pot}	C/chlor *a*	Chlor *c*/*a*	K_b/chlor
μ_{pot}	0.51*				
C/chlor *a*	0.25	−0.59**			
Chlor *c*/*a*	0.17	0.05	0.20		
K_b/chlor *a*	−0.46*	−0.19	−0.14	0.20	
K_b/C	−0.50*	−0.09	−0.32	0.20	0.92**

pected because of the higher respiratory needs of this class.

Another point of interest is the distinctive vertical distribution of the C/chlor ratio during weeks F and N (Fig. 13). The maximum ratio at 2 m depth suggests that the dinoflagellates are concentrated at or near this depth and thus they can maintain a desired depth distribution against the dissipative forces of vertical eddy diffusivity as has been demonstrated in Chesapeake Bay (Seliger et al. 1975).

Two sampling days, I and M, have markedly higher μ_{pot} values (Fig. 11), both periods being dominated by *Melosira* sp. Again, assimilation numbers were intermediate. The single high value for assimilation number (cruise S) was associated with a high C/chlor ratio (160), low biomass, and Chrysophyte flagellates. This combination of high C/chlor ratios, low biomass, and high assimilation numbers was observed during the middle of a 3½-d fixed station and again the samples were high in Chrysophyte flagellates (Sinclair 1977). This parallelism suggest that high C/chlor ratios are associated with the flagellates rather than due to heterotrophic "contamination" of the ATP samples.

The importance of these observations is that, with a combination of ATP, chlorophyll *a*, and primary production (and the derived parameters) one can have a much more detailed image of the character of the mixed phytoplankton assemblage than is possible with just chlorophyll and primary production measures. In addition, all the major features of the summer distribution of θ, μ_{pot}, and C/chlor appear to be due to changes in species composition rather than temporal differences in physiological state or temperature effects. This relationship between the distributions of ATP-derived parameters and species composition is supportive evidence that heterotrophic "contamination" is not of sufficient magnitude to mask the phytoplankton contribution.

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Myxosoma cerebralis: Detection of Circulating Antibodies in Infected Rainbow Trout (*Salmo gairdneri*)

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Circulating antibody against spores of *Myxosoma cerebralis* has been detected for the first time in rainbow trout (*Salmo gairdneri*) affected with whirling disease. An indirect fluorescent antibody test (IFAT) was employed for detection of trout immunoglobulin. Most sera of fish from an infected experimental group were found to give a positive reaction in the IFAT, while the majority of specific-pathogen-free control sera gave no reaction. Reagent preparation as well as staining and evaluation procedures are given. These results provide additional information for understanding the host/parasite relationship and offer a potential for development of a nondestructive serological test for whirling disease.

Key words: indirect fluorescent antibody test, immune response, whirling disease

GRIFFIN, B. R., AND E. M. DAVIS. 1978. *Myxosoma cerebralis*: detection of circulating antibodies in infected rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 35: 1186–1190.

Nous avons décelé pour la première fois chez des truites arc-en-ciel (*Salmo gairdneri*) affectées de la maladie giratoire un anticorps en circulation contre des spores de *Myxosoma cerebralis*. Pour la détection de l'immoglobuline des truites, nous avons utilisé un test d'anticorps à fluorescence indirecte (IFAT). Nous constatons que la plupart des sérums de poissons provenant d'un groupe infecté expérimentalement ont une réaction positive à l'IFAT, alors que la majorité des sérums de témoins libres de l'agent pathogène ne donnent aucune réaction. Nous décrivons la préparation des réactifs et les méthodes de coloration et d'évaluation. Ces résultats nous permettent de mieux comprendre la relation hôte/parasite et pourraient servir à mettre au point un test sérologique non destructeur de la maladie giratoire.

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THE myxosporidan parasite *Myxosoma cerebralis* causes the disfiguring, often crippling or fatal whirling disease of salmonids. The pathobiology of whirling disease has been reviewed most recently by Halliday (1976).

Lom (1969) suggested that poikilothermic animals generally show little, if any, response to myxosporidan infections. Neither Pauley (1974) nor Halliday (1974) was able to detect anti-*M. cerebralis* antibody in rainbow trout (*Salmo gairdneri*) through the use of serological techniques. Pauley's (1974) investigation of the spore antigens of *M. cerebralis* indicated that they mimic the antigens of rainbow trout cartilage even though electrophoretic differences were demonstrated. This host antigen mimicry apparently would account for the ease with which the parasite establishes itself in host tissues.

Halliday (1974), using rabbit antibody against whole trout serum in an indirect fluorescent antibody test was unable to detect specific antibody in rainbow trout with natural infections or in trout experimentally inoculated with spores of *M. cerebralis*.

The ability of various fish species to develop immunity and demonstrable antibody during some parasitic infections has been documented. Hines and Spira (1973) demonstrated acquired immunity as well as the presence of immobilizing antibodies in common carp (*Cyprinus carpio*) infected with *Ichthyophthirius multifiliis*. Molnar and Berczi (1965) detected precipitating antibodies to *Ligula intestinalis* in infected bream (*Abramis brama*). Other investigators have shown the presence of an immune response, indicated by increased levels of resistance to challenge (although not demonstrated by

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the presence of specific antibody) in several species: Kennedy and Walker (1969) in dace (*Leuciscus leuciscus*) infected with *Caryophyllaeus laticeps*; Beckert and Allison (1964) in white catfish (*Ictalurus catus*) infected with *Ichthyophthirius multifiliis*; and Nigrelli (1937) in Florida pompano (*Trachinotus carolinus*) and permit (*T. falcatus*) infected with *Epibdella melleni*.

The purpose of this paper is to report the development of an indirect fluorescent antibody technique for detection of trout antibodies to *M. cerebralis*, and to show by application of the technique a correspondence between the presence of *M. cerebralis* spores in fish tissue and specific antibody in their serum.

Materials and Methods

General Approach

Rainbow trout immunoglobulin was purified by elution from specific antigen–antibody complexes and used as antigen for the production of anti-trout immunoglobulin antisera in rabbits. Rabbit immunoglobulin G was purified by standard methods and labeled with fluorescein isothiocyanate for use in conventional indirect fluorescent antibody tests.

Infected and Specific-Pathogen-Free Rainbow Trout

Rainbow trout infected with *M. cerebralis* were obtained from the Lamar (Pennsylvania) National Fish Hatchery, and specific-pathogen-free rainbow trout controls from the Leetown (West Virginia) National Fish Hatchery. Fish were kept in 12°C spring water, the chemical characteristics of which were reported by Warren (1963).

Preparation of Antiserum

Immunoglobulin from high titer rainbow trout antiserum against *Aeromonas salmonicida* was purified by a modification of methods described by Cisar and Fryer (1974). Adult rainbow trout (25–30 cm fork length) were immunized by intraperitoneal inoculation of 0.5 mg (dry weight) of formalin-killed *A. salmonicida* in Freund's incomplete adjuvant, followed in 6 mo by an intraperitoneal inoculation of 1 mg of a similar bacterial preparation in phosphate-buffered saline, pH 7.2. The fish were bled 4 wk after the second injection and the sera from 30 animals were pooled.

The globulin fraction was collected after precipitation with an equal volume of cold saturated ammonium sulfate and centrifugation at 27 000 × *g* for 10 min at 4°C. The globulin proteins were dialyzed against two changes of 0.1 M phosphate buffered saline, pH 7.2 (globulin: buffer ratio = 1:100), and specific immunoglobulins were adsorbed for 1 h at 25°C onto formalin-fixed *A. salmonicida* cells (50 mg dry weight/mL serum). The bacterial cells had been washed with 0.1 M KCN, pH 11.0 to eliminate any bacterial components that might later be eluted along with the specific antibody. The cells were then equilibrated in 0.1 M phosphate buffered saline, pH 7.2. After immunoglobulin adsorption, cells were centrifuged at 27 000 × *g* for 10 min at 4°C. The supernatant was discarded and the cells were resuspended, washed, and centrifuged three times in 0.1 M phosphate buffered saline, pH 7.2. Immunoglobulins were then eluted from the cells by three sequential resuspensions and centrifugations in 0.1 M KCN; the volume of each wash was equal to that of the original serum. Immunoglobulins were precipitated from KCN by the addition of an equal volume of cold saturated ammonium sulfate, collected by centrifugation, and dissolved in and dialyzed against 0.1 M phosphate buffered saline, pH 7.2 at 4°C.

Immunoglobulin samples were applied to a 1.4- × 100-cm column of Sephadex G-100 (Pharmacia Fine Chemicals, Inc.) equilibrated in 0.1 M phosphate buffered saline, pH 7.2. The leading portion of the effluent void volume peak absorbing at 280 nm was collected and concentrated (by the addition of saturated ammonium sulfate and centrifugation) as before. After exhaustive dialysis against 0.1 M phosphate buffered saline, pH 7.2, the protein concentration was adjusted to 2 mg/mL. For quantitation, protein concentration was estimated on the basis of optical density, assuming an extinction coefficient of 1.6/mg/mL at 280 nm.

The immunoglobulin was emulsified with an equal volume of Freund's incomplete adjuvant. Rabbits were immunized by injection of 1.0 mL of the adjuvant mixture divided between two subcutaneous sites. A subcutaneous booster of 1 mg of protein in 1 mL of 0.1 M phosphate buffered saline, pH 7.2, was given 20 d later. Final bleeding followed 7 d after the booster. Rabbit anti-rainbow trout immunoglobulin prepared in this way gave a single line of precipitation in immunodiffusion against whole rainbow trout serum.

Fluorescent Labeling

Fluorescein isothiocyanate was conjugated to rabbit antibody by a modification of the methods of Johnson and Holborow (1973).

Rabbit antibody to rainbow trout immunoglobulin was mixed with an equal volume of cold, saturated ammonium sulfate; after centrifugation the precipitate was dissolved in and dialyzed against 0.015 M phosphate buffer, pH 8.0, and applied to a column of Sephadex DEAE, A-25 (Pharmacia Fine Chemicals, Inc.), equilibrated in the same buffer. Nonbinding protein was collected, then concentrated by adding an equal volume of saturated ammonium sulfate followed by centrifugation at 27 000 × *g* for 10 min at 4°C. The precipitate was dissolved in and dialyzed against 0.15 M NaCl and the protein concentration was adjusted to 10 mg/mL. Protein concentration was estimated on the basis of optical density, assuming an extinction coefficient of 1.4/mg/mL at 280 nm.

A 10% volume of 0.5 M carbonate–bicarbonate buffer, pH 9.5, was added and dry powdered fluorescein isothiocyanate (Isomer I, Sigma #F7250, Sigma Chemical Co.) was added at a ratio of 40 μg/mg protein. The mixture was shaken gently for 2 h at room temperature (pH maintained at 9.5 by addition of 0.5 M Na_2CO_3), and then applied to a 2.5 × 12 cm column of Sephadex G-25 equilibrated in 0.1 M phosphate-buffered saline, pH 7.2, for removal of unreacted dye. The fluorescein to protein ratio ranged from 0.4 to 1.1 in different preparations as indicated by absorption at 495 nm/280 nm. When a counterstain was used, Rhodamine (Difco #2340-56) was added at a 1:20 ratio. Conjugated rabbit anti-rainbow trout immunoglobulin antibody prepared in this way retained its biological activity and gave a single line of precipitation in immunodiffusion against whole rainbow trout serum.

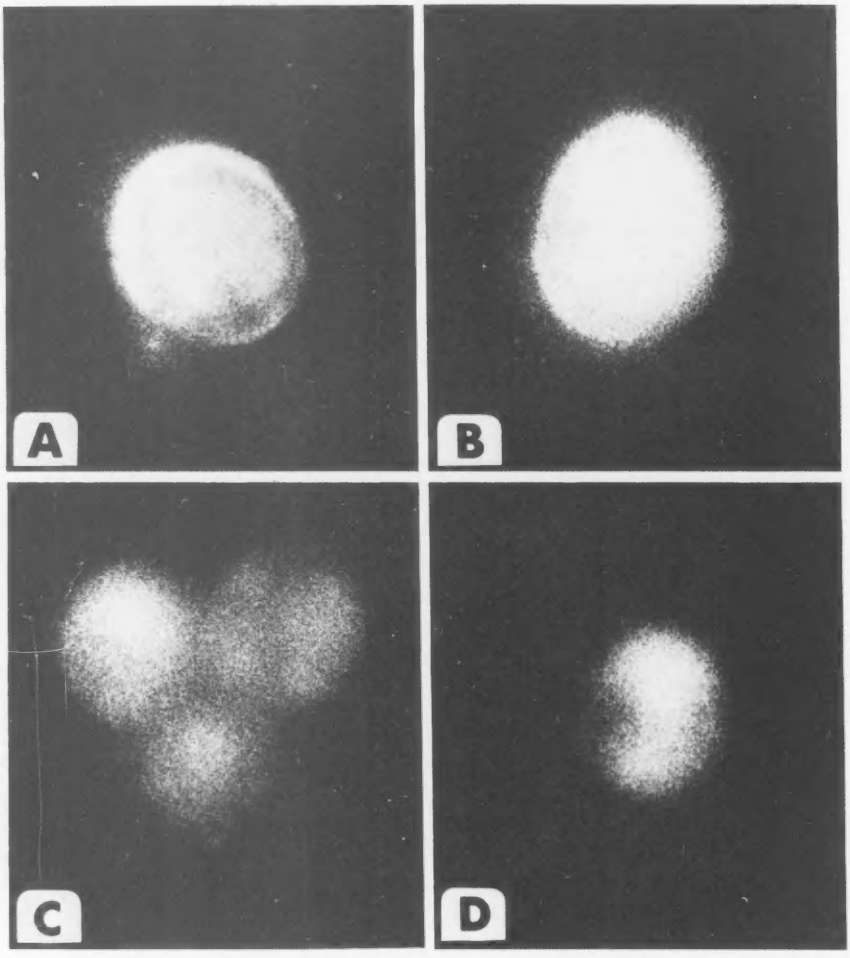

FIG. 1. Mature spores of *M. cerebralis* in the indirect fluorescent antibody test. A, spore after staining, using infected trout serum; B, spore after staining, using strongly positive, undiluted, infected trout serum; C, spores after staining, using serum from specific-pathogen-free fish; D, unstained spore, showing autofluorescence.

INDIRECT FLUORESCENT ANTIBODY STAINING

For the test, spores of *M. cerebralis* obtained from infected trout by the pepsin–trypsin–dextrose method (Markiw and Wolf 1974a) were spread on an acid cleaned slide and dried (60°C for 10 min). Test rainbow trout serum was added and slides were incubated 40 min at room temperature in a moist chamber. Slides were rinsed with a stream of 0.1 M phosphate buffered saline, pH 7.2, and immersed in the same buffer and shaken gently for 30 min at room temperature. The conjugate was added and after incubation for 30 min the slides were rinsed and washed as before. Stained slides were mounted in a mounting medium of 50:50 glycerol, 0.5 M carbonate–bicarbonate buffer, pH 9.5, and observed with reflected UV illumination at 500×.

To assess correlation between the presence of spores and specific antibodies, we microscopically examined an aqueous preparation of crushed cranial cartilage and bone from each fish for spores; the time limit was 5 min per sample.

Results

The indirect fluorescent antibody test revealed a correlation between the presence of spores in fish tissues and the presence of serum antibody against *M. cerebralis*. Sera from the group of fish infected with *M. cerebralis* gave fluorescence in 16 of 18 samples when tested undiluted and in 14 of 18 when diluted 1:3.

Spores were detected in 14 of the 18 trout when crushed cranial cartilage smear preparations were ex-

amined microscopically. There was not an absolute correlation between the presence of spores and the presence of antibody in individuals since in one case spores were detected but antibodies were not demonstrable in the serum of that animal and conversely antibody was detected in three sera from specimens in which spores were not detected. However, application of the sign test showed that the association of spores with serum antibody was statistically significant ($P = 0.01$). The undiluted control sera, on the other hand, yielded no fluorescence in 15 of 18 individual samples and when diluted 1:3 gave weak fluorescence in only one. Spores were not detected in the crushed cranial smears prepared from these specific-pathogen-free controls.

The test was specific for trout antibody to *M. cerebralis*. If spores were incubated with positive serum and then reacted with unconjugated rabbit anti-trout immunoglobulin before being stained with conjugate, specific fluorescence was not observed. Spores incubated with high-titer rainbow trout anti-*A. salmonicida* sera and then stained with the conjugate were also negative.

Fluorescence was usually apparent along the periphery of the spore with little staining of internal structures (Fig. 1A). Some undiluted sera gave bright confluent fluorescence over the entire spore surface (Fig. 1B); however, when these sera were diluted 1:3 the staining pattern was peripheral (as in Fig. 1A). Spores treated with sera from normal controls typically showed a dull autofluorescence after staining (Fig. 1C) that was characteristic of unstained spores (Fig. 1D). Polar filaments were often observed in the stained preparation but no consistent staining pattern was noted. The best fluorescence was obtained with conjugate preparations exhibiting a fluorescein to protein ratio ranging from 0.7 to 1.1. Rhodamine was not necessary since negative and positive preparations were readily distinguishable; however, the counterstain facilitated the identification of the bicapsular spore.

Discussion

The demonstration of circulating antibody in the present study does not support currently held opinions that rainbow trout do not mount an immune response to *M. cerebralis*. Halliday (1974) has suggested that the spores of *M. cerebralis* may occupy an immunologically privileged or guarded site in the cranial cartilage and bone of the infected animal, or alternatively that the absence of detectable antibody may be due to what he described as "non-pathogenicity of the spores." Failure to detect antibody in infected fish appears to have been the result of Halliday's use of a nonspecific fluorescent antibody conjugate rather than due to an insufficiency on the part of the infected animal. Similarly, Pauley's (1974) failure to demonstrate the presence of antibody may well have been due to the use of an insensitive assay — immunodiffusion in gel — rather than to the absence of antibody resulting from antigenic mimicry. Indeed, Pauley clearly illustrated molecular differences between spore material and normal fish tissue by electrophoretic analysis.

The detection of an immune response in trout infected with *M. cerebralis* provides additional background for understanding this host/parasite relationship. Dineen (1963a, b) suggested that specific antigenic disparity between parasite components and host tissues can serve to stabilize the relationship. The immune response to a limited range of parasitic antigens would control the parasite population to within tolerable limits that would not kill the host, thus enhancing survival of the parasite. Any strong display of critical parasite antigens which might lead to elimination of the infection through an intense host immune response would work against the long-term survival of the parasite. Antigenic disparity then, to best serve the host/parasite relationship, must be reduced but not eliminated.

Although the rainbow trout is not considered to be the natural host for *M. cerebralis* (Hoffman 1970), it is in the same genus as the natural host, the brown trout (*Salmo trutta*). The detection of antibody to *M. cerebralis* in infected rainbow trout makes plausible the argument of limited antigenic disparity in explaining the stability of the natural host/parasite relationship.

The inability of previous investigators to detect the immune response of trout infected with *M. cerebralis* has prevented consideration of a serodiagnostic test that is independent of finding spores for diagnosis, hence the methods of spore detection have been refined considerably. Markiw and Wolf (1974b) compared several of the currently available methods of detecting spores in an infected population and concluded that the pepsin–trypsin–dextrose centrifugation method (Markiw and Wolf 1974a) was most sensitive. The demonstration of circulating antibody to *M. cerebralis* spores in infected rainbow trout, as reported here, opens for investigation the use of the indirect fluorescent antibody test, or other serodiagnostic techniques, in the diagnosis of the disease. This test is nondestructive and can be performed in a matter of 2 to 3 h.

A number of factors required for a useful serodiagnostic test employing the indirect fluorescent antibody test are still to be investigated. These include an analysis of the range of cross-reactivity with other myxosporidans; a study of the age of the fish when antibody is detectable; an understanding of the influence of temperature on production of antibody; and an understanding of the predictive value of specific antibody as an indicator of the presence or absence of infection.

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Acute Effects of Underwater Construction Blasting on Fishes in Long Point Bay, Lake Erie

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TELEKI, G. C., AND A. J. CHAMBERLAIN. 1978. Acute effects of underwater construction blasting on fishes in Long Point Bay, Lake Erie. J. Fish. Res. Board Can. 35: 1191–1198.

We detonated 201 blasts 22.7–272.4 kg per charge with a high explosive (Hydromex) in 4–8 m of water at Nanticoke, Lake Erie. Shortly after each blast turbidity and CO_2 concentrations increased markedly, returning to ambient levels within 3 h of the blast. Postblast pressure and charge weight were directly related ($r = 0.70$, $P < 0.05$). Fatality radii ranged from 20 to 50 m for 22.7 kg per charge to 45–110 m for 272.4 kg per charge. Common blast-induced injuries were swimbladder ruptures and hemorrhaging in the coelomic and pericardial cavities. Laterally compressed species were more susceptible to blast pressure gradients than were the fusiform fishes. Temporally, surface mortalities were high only in the spring and late summer. Spatially, 47% of the total blast mortality was not visible from the water surface.

Key words: blasting impact, hydraulic pressure gradients, fish mortality, species specific susceptibility

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On a fait détonner 201 charges d'un puissant explosif (Hydromex) à raison de 22.7 à 272.4 kg par charge dans 4 à 8 m d'eau à Nanticoke, lac Erié. Peu après chaque détonation, la turbidité et les teneurs en CO_2 augmentent notablement, pour retourner à des niveaux ambients en dedans de 3 h après l'explosion. Il y a corrélation directe entre la pression d'après explosion et le poids de la charge ($r = 0.70$, $P < 0.05$). Le rayon de mortalité varie entre 20 à 50 m pour 22.7 kg par charge et 45–110 m pour 272.4 kg par charge. Les blessures les plus communes résultant des explosions sont des ruptures de la vessie gazeuse et des hémorragies dans les cavités coelomique et péricardiaque. Les espèces à corps comprimé latéralement sont plus sensibles aux gradients de pression causés par les explosions que les poissons fusiformes. Dans le temps, les mortalités à la surface ne sont élevées qu'au printemps et à la fin de l'été. Dans l'espace, 47% des mortalités totales sont invisibles à la surface de l'eau.

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SUBMARINE blasting is practiced throughout the world (C.I.L. 1972). Its principal applications are in seismic exploration for oil and gas and the maintenance or construction of waterways and harbors. Although extremely difficult to quantify, Falk and Lawrence (1973) stated that 51 256 kg of explosives were detonated during only seven blasting operations in 1972. They estimated that in the Northwest Territories some 165 operations were completed exposing many hundreds of square kilometres of water to blasting impacts.

Detonations may be classified by their final intent, i.e. either geological, when charges are suspended in the water or resting on the bottom, or construction, when explosives are placed in drilled holes. Physical effects of the former have been extensively documented (Hubbs and Rechnitzer 1952; Falk and Lawrence 1973). Thomas (1958) and the Ontario Ministry of Transportation and Communications (1974) reported on the acute effects of construction blasting on fishes. Their results proved inconclusive as either the detonations were of low magnitude or too few fish were exposed to the blasts.

During the summer of 1975 the Steel Company of Canada initiated an extensive blasting program designed to increase the depth of nearshore waters fronting their property at Nanticoke, Lake Erie. More than 200 detonations provided the basis for this experiment, namely, to determine the acute effects of blasting on fish and to quantify the principal components of an underwater explosion that control the magnitude of the biological impact. To that end this paper will emphasize the re-

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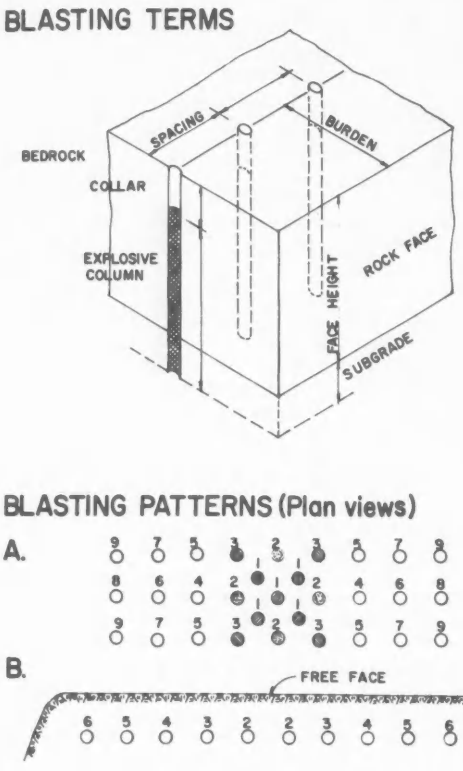

FIG. 1. Blasting terms and typical sinking cut (A) and production cut patterns (B) used in the construction blasting at Nanticoke, Lake Erie. Numbers represent detonation sequence. Time between ignition of charges 2, 3, 4 etc. measured in milliseconds, is termed the delay.

lationship of charge type and size to species specific injuries and deaths as well as providing equations for the estimation of mortality radii for given charge weights.

Materials and Methods

BLASTING METHODS AND AGENTS

As drill hole preparation and the type of explosive used profoundly affect the detonation impact, a brief description of the techniques and blasting agents follows. Three methods were applied to fragment the limestone bedrock and loosen the glacial till and clay overburden. "Sinking cut" charges (Fig. 1), requiring the greatest detonation energy, blasted holes into the bedrock exposing an open rock face for "production" blasting. The production blasts simply sliced away and crushed sections of the rock face. "Toe" blasts were used to level high spots left after the other blasting had been completed. Of the three types of detonations the toe blasts are the most unpredictable as their charge size is frequently overestimated to ensure that the rock is removed to a specific elevation. The sinking and production blast charges are precisely determined, preventing the generation

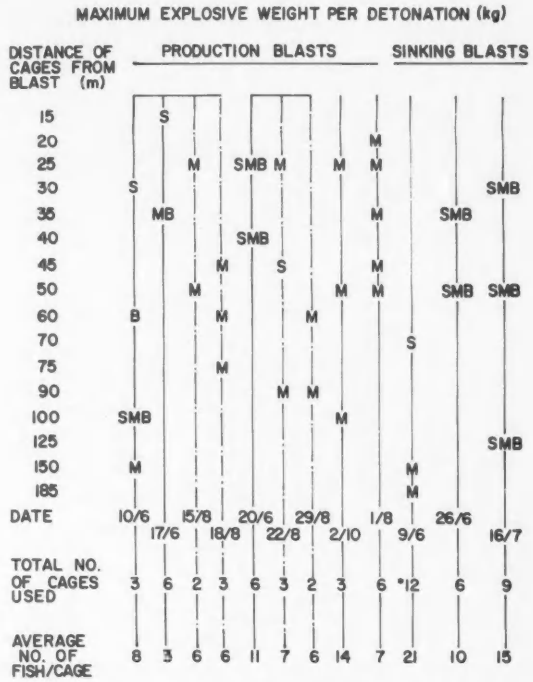

FIG. 2. Number of fish used per cage and their distance from the underwater blasts in rock (————) and rock and clay (—— . ——) bottom types in Long Point Bay, Lake Erie, 1975. Dates are expressed as day/month; '*' nine cages set 200–1006 m from blast; S — Surface, M — Midwater, and B — Bottom.

of large shock waves. The "collar" (Fig. 1) and the density of the material to be blasted add additional variability to the blast pressures. The longer the collar and the denser the material the less energy will be vented.

The rate at which a blasting agent ignites, termed the detonation velocity (DV), its distinctive characteristic, ranges from 1709 $m \cdot s^{-1}$ for black powder to specially formulated trinitrotoluene (TNT) at 7625 $m \cdot s^{-1}$ (C.I.L. 1972). Explosives used in this study were Canadian Industries Ltd. (C.I.L.) Hydromex M210 and T3 with DV's of 4938 and 5486 $m \cdot s^{-1}$, respectively (C.I.L. 1972).

Dissolved CO_2, pH, O_2, NO_3 and turbidity were measured before and after every sinking cut blast, using a portable water quality analysis kit.

CAGED FISH

Fishes to be exposed to the blasts were caught in trap and seine nets that were fished well beyond (3 km) the blast effect zone. Within 24 h of capture fish were placed in 2.3 × 1.1 cm wire-mesh cages with two compartments each measuring 30 × 40 × 60 cm. To reduce predation and territorial stress we placed the smaller (100–200 mm) and larger (201–400 mm TL) fish in separate compartments. The number of cages per blast varied according to the availability of fish (Fig. 2). To monitor the physical effects of cage confinement we placed a minimum of two fish per

size-class per species in cages anchored beyond (3 km) the blasting zone at the same time that other samples were being exposed to the detonation. In addition, all caged fish were inspected for holding mortalities 0.5 h before each blast.

We assumed that the pressure gradient generated by the sinking cut blasts would be omnidirectional; therefore the cages were placed along three axes 120° apart emanating from the blast center. For all production blasts cages were placed in a line perpendicular to the rock face assumed to be the path of peak pressure for this type of blasting pattern.

Immediately after each blast all cages were examined and the dead fish, plus those exhibiting an obvious loss of equilibrium or having a visible injury, were prepared for autopsy. Any seemingly unaffected fish were transferred, with a counterpart from the control cages, to a holding tank for short-term ($\leq$290 h) observations after which time they too were dissected. After all sinking cuts some of the healthy caged fish were kept in the blast area for 24 h to determine whether the blast-induced water quality changes had any acute effects. The varying degrees of blast-induced injuries were classified on a scale from 1 to 7 based on our autopsies as well as the findings of Hubbs and Rechnitzer (1952) and Ferguson (1962).

Internal Injury Index

Code	Type of injury	Survival time (h) Min	Max
1	slight hemorrhaging of arteries	290	
2	lining of swim bladder stretched — fibrous connective tissue ruptured	12	290
3	swim bladder with pinhole-size rupture	2	72
4	1 + 2	2	49
5	heart, kidney, and/or associated organs hemorrhaged	?	33
6	ribs protruding into body cavity or swim bladder severely ruptured	0	1
7	6 plus multiple hemorrhaging	0	0.5

Free-Swimming Fish

Mortalities of free-swimming fish were assessed by boat surveys. The surveys were made within 30 min of each detonation along two transect lines crossing over the blast center. The mean distance from the epicenter to the perimeter where no dead or stressed fish were seen on the surface was termed the fatality radius (FR). All visibly stressed or dead fish were counted in 10–20 m^2 randomly selected plots yielding a mean number ($\bar{m}$) of fatally injured fish per square metre. To assess subsurface mortalities we conducted seven midwater and five bottom tows using 565-μm mesh, 1.0- and 0.5-m conical nets. Conservative estimates of the density of live and dead fish per cubic metre of water were calculated from these net-haul data using the equation

$$M = Fv\left(d\frac{Nf}{N}\right)$$

where M equals the estimated number of fish killed within the sampling stratum, Nf equals the number of dead or dying fish collected from the net tows, N equals the total (both alive and dead) number of fish sampled, and d equals their density per cubic metre of water. By summing the M over a number of strata and adding this to the surface counts the total fish kill was estimated.

Pressure Recording and Calculations

Blast pressures were monitored by three-channel pressure sensitive hydrophones attached to a number of fish cages at varying depths and distances from the blast. Pressure readings obtained from the three hydrophones were recorded as light traces on one strip of unexposed film (McAnuff and Booren personal communication). As each hydrophone was calibrated to a particular pressure range the traces could be easily interpreted. Pressure readings at nonmonitored cages were estimated using Cole's (Cole 1948) equation for peak pressure, where P equals pressure in kilopascals (pounds per square inch $\times$ 6.896) and W equals the maximum

$$P = f_2\left(\frac{W^{1/3}}{R}\right)^{1.14}$$

amount (in kilograms) of explosive detonated per charge, R equals the distance in metres from the blast center to the point for which P is to be calculated, and f_2 is an empirically derived constant describing the relationship of pressure to charge weight and distance. A set of readings providing P, W, and R are needed before a reliable f_2 constant can be generated by solving for f_2 in the original Cole equation. By solving for R we were able to estimate a blast fatality radius (FR).

Results

Blast Pressure Readings and Water Quality Changes

From June 9 through October 1, 1975 three sinking cuts, 67 rock, and 127 clay–till production blasts were detonated. Pressure readings from 3 sinking cuts, 7 rock blasts, and 18 clay–till blasts were recorded (McAnuff and Booren personal communication). The denser the material to be blasted, and the deeper the collar, the more rapid was the decay of the pressure front emanating from the blast (Fig. 3).

A 38-kg rock blast monitored from 24 m away produced 26 kPa while a 30-kg charge detonated in clay–till material developed 93 kPa at 25 m. For the 55 readings pressures ranged from 8.61 kPa for a 90.8-kg charge at 73 m to 158 kPa for a 45.4-kg charge measured from 14 m away.

The water quality analysis revealed changes in free CO_2 and turbidity, which persisted for 52–122 min, respectively, after detonation. Free CO_2 increased from 11.5 to 25 and 18.6 to 24.0 $mg \cdot L^{-1}$ during the June 9 and July 2 detonations, respectively. Turbidity increased by a few units. Regular readings of five variables, for the next 4 mo, indicated no progressive or latent changes in the water quality of the blast zone.

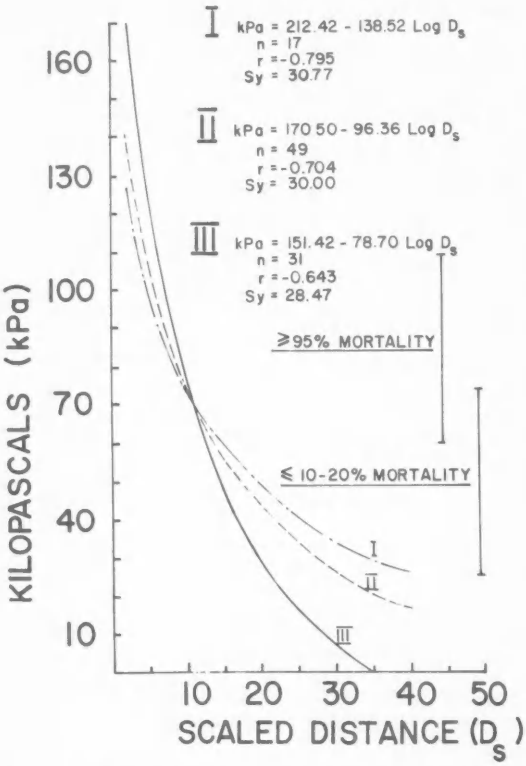

FIG. 3. Relationship of substrate type; Rock (I), All material (II), Till and clay (III); to the rate at which the blast pressures decay. D_s equals $(R/W^{1/3})^{1.14}$ from Cole's (1948) formula. Mortality lines indicate the mean kilopascals at which fish deaths were recorded; 95% mortality = 87.75, SD = 24.42; 10–20% mortality = 52.77, SD = 23.28.

TABLE 1. Pressure at which caged fish exposed to the construction blasting received lethal injuries, Long Point Bay, Lake Erie, May–October, 1975. Minimum lethal pressure, (MLP) = 10–20% mortality; fatal pressure (FP) = 95% mortality.

Species	Number autopsied	MLP (kPa)	FP (kPa)
Lepomis gibbosus	40	30	69
Pomoxis sp.	44	30	73
Morone chrysops	19	30	88
Dorosoma cepedianum	3	39	?
Perca flavescens	91	40	73[a]
Micropterus dolomieui	11	65	?
Ambloplites rupestris	79	65	73
Aplodinotus grunniens	9	73	?
Carpiodes cyprinus	23	76	76
Catostomus commersoni	7	73	?
Ictalurus natalis	51	73	?
Salmo gairdneri	18	85	100
Cyprinus carpio	2	7	150

[a]One yellow perch possessed no internal injury after being exposed to 150 kPa pressure.

EFFECTS OF BLASTS ON EXPOSED, CAGED FISHES

Of the 397 fish examined 33 were alive before being autopsied. Based on the fish held for observations, an injury index greater than one was usually fatal. Injury type 1 was found in control as well as test fishes and was attributed to handling stress and cage confinement. Head and fin abrasions plus occasional capillary ruptures around the fins were signs of handling damage. Common recurring fatal injuries were types 3 and 5. Injuries to the swim bladder were easily identified and in most cases the organ appeared to have burst outward. Pressures associated with a type 2 injury (30–85 kPa) were termed minimum lethal pressures (MLP) as from 10 to 20% of the exposed fish were killed at those levels (Table 1). Beyond the type 2 injury all pressures (69–150 kPa) were grouped as fatal pressures (FP) as ≥95% of the exposed fish died at these levels. Pumpkinseed (*Lepomis gibbosus*), crappie (*Pomoxis* sp.), and white bass (*Morone chrysops*) were the most sensitive to pressure changes while the most resistant species were rainbow trout (*Salmo gairdneri*), yellow bullhead (*Ictalurus natalis*), and white sucker (*Catostomus commersoni*) (Table 1 and Fig. 4). Sublethal injuries to fishes at explosive loads ≤275 kg occurred from 50 to 115 m from the blast center, whereas death was certain for fish <50 m from the blast (Fig. 4). Among the exposed yet externally undamaged fish held for observations, impending death was preceded by a loss of equilibrium, reduced swimming activity, and finally long periods (>1 h) of lying motionless on the bottom of the holding tank.

EFFECTS OF BLASTS ON FREE-SWIMMING FISHES

After each of the nine blasts surveyed, progressively fewer visibly injured or dead fish were observed floating on the water surface. The emerald shiner (*Notropis atherinoides*) was the only species collected consistently throughout the blasting period and was particularly prominent during July when their young-of-the-year began appearing in the samples. Trout-perch (*Percopsis omiscomaycush*) and rainbow smelt (*Osmerus mordax*) were killed only during the June 9 blast. The species composition of the fish samples we collected decreased from six in June to only emerald shiner on October 2 (Table 2).

Subsurface net-tows were conducted on June 17 and October 2. The June tows yielded only five fish while many more were captured on the later date (Table 3). During the October survey 50% of the netted fish were alive as the catch was being counted. A subsample of these undamaged fish remained alive beyond the 290-h holding period. Higher mortalities were observed in the midwater to surface stratum (<4 m) than for deeper strata (Table 3). Tow data revealed that 40% of the emerald shiners in 7 m of water and 100% of the

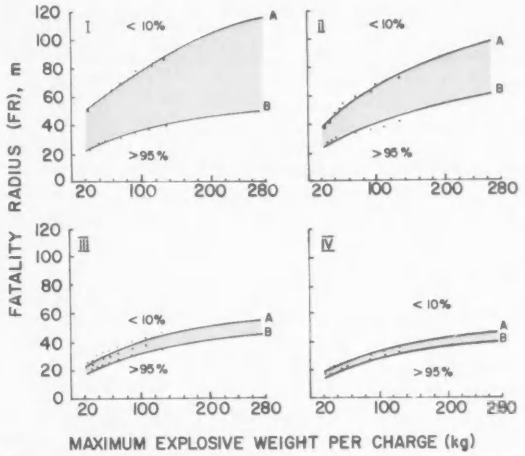

FIG. 4. Relationship of kilogram per charge to fatality radii (FR): A = 10–20% mortality, B = 95% mortality. Number of data pairs = 10, $r^2 = 0.99$, and Sy = 0.003. In equations for III n = 20, $r^2 = 0.981$, and Sy = 0.21. IA, Log FR = 1.2423 + 0.3340 Log kg; IB, Log FR = 0.8814 = 0.3390 Log kg; IIA, Log FR = 1.3340 = 0.3337 Log kg; IIB, Log FR = 0.9087 + 0.3323 Log kg; IIIA, Log FR = 0.9261 + 0.3344 Log kg; IIIB, Log FR = 0.8199 + 0.3429 Log kg; IVA, Log FR = 0.8465 + 0.3382 Log kg; IVB, Log FR = 0.7297 + 0.3624 Log kg. I = pumpkinseed, crappie, white bass; II = rock bass, smallmouth bass, yellow perch, freshwater drum; III = quillback, white sucker, yellow bullhead; IV = rainbow trout.

alewives and smelt in 2.5 m of water were killed by the blasts. The mortality within the entire water column did not exceed 78%. The total subsurface mortality was estimated to be 30 204 fish compared with 33 134 surface deaths.

Discussion

Based on our findings and those of others (Hubbs and Rechnitzer 1952; Falk and Lawrence 1973) the lethality of an explosive is directly related to its detonation velocity. Hubbs and Rechnitzer (1952) found that fish were immune to slowly detonating black powder and were able to withstand pressures of 854–1103 kPa. Using dynamite the MLP was reduced to 276 kPa. High explosives used in our experiments, having a DV 1.5 times (4900 $m \cdot s^{-1}$) that of dynamite, lowered the lethal range to between 30 and 150 kPa. The more rapid the detonation velocity the more abrupt was the resultant hydraulic pressure gradient and the more difficulty fish had adjusting to the pressure changes. Clearly a knowledge of the DV is critical to a true understanding of the impact of blasting on fish. During our study most explosions were in a rock or clay substrate; therefore they produced low-level overpressures. The fatality radii from these charges that weighed between 22 and 272 kg were 20–114 min. There were significant differences in the pressure decay rates for detonations in bedrock and clay (Fig. 3).

Injuries incurred by free-swimming and caged fish were similar to those reported by Ferguson (1962), Kearns and Boyd (1965), and Paterson and Turner (1968). Common injuries viz. hemorrhaging in the coelomic or pericardial cavity and swim-bladder ruptures were not only a function of the compression but also the rarefaction portion of the pressure wave. The swim bladders from many of the autopsied fish had burst outward indicating a negative pressure effect.

Differences in species specific susceptibility to blast injuries were probably a function of the fish's shape and swim-bladder formation. Pumpkinseed, white bass, and crappie, all physoclistic, were the most sensitive to the blast (Fig. 4). Their laterally compressed bodies exposed a great deal of surface area to any oncoming pressure front. Mortality within this group was highly variable due also to body shape. The orientation of a laterally compressed body head-on into the pressure front presented a much smaller target than a fish positioned at some other angle to the shock wave. Rainbow trout, white suckers, and yellow bullheads, physostomes with fusiform bodies, were the least affected by the blast. Fitch and Young (1948) observed that fish with cylindrical bodies were more resistant to pressure gradients than were those with larger surface to volume ratios. Muth (1966) concluded that of the fish species he exposed to blasts the northern pike (*Esox lucius*), a fusiform fish, was the least sensitive to rapid pressure changes. These observations support our contention that the rounder the fish the less will be the overall impact of blast pressures and they also suggest that fish with a large body size are not necessarily more susceptible to injury than are smaller ones.

Observable changes in behavior, e.g. swimming with a fluttering movement in an erratic pattern or swimming on one side, the result of exposure to a sublethal blast, rarely lasted for more than 1 h after the detonation. However, these stunned fishes especially those near the surface suffered heavy losses through predation, principally by herring gulls (*Larus argentatus*). In fact predation was so effective that many of our surface mortality counts were rendered useless by the large flocks of gulls congregating over the blast site after the first warning whistle, then descending on any visible fish within seconds of a blast.

Fitch and Young (1948), Coker and Hollis (1950), and the Ontario Ministry of Transportation and Communications (1974) found that from 11 to 50% of all fish killed by blasts sank to the bottom. Our sampling with a 1.0–0.5 m net was the only, albeit crude, way to assess subsurface mortality. The 47% subsurface mortality estimates calculated from the October tows (Table 3) may be low, as injured fish well enough to swim could have avoided the net, but judging from the high density of fish captured (Table 3) we feel that escapes were minimal. The high turbidities in the nearshore waters of Long Point Bay also aided in reducing

TABLE 2. Surface mortality estimates of free-swimming fish exposed to construction blasting at the Stelco dock, Long Point Bay, Lake Erie, 1975. Kilopascal (kPa) = 0.145 lb/in^2.

Date	Maximum kg/delay	Material blasted	No. of holes	Species	Mean no. dead and stressed fish/m^2	Estimated mean fatality radius (m)	Estimated total mortality	Estimated pressure at perimeter of fatality radius (kPa)
June 9	66.28	Bedrock	20	Rainbow smelt	9.0	20	11 304	128
				Emerald shiner	0.5		628	
				Trout-perch	1.0		1 256	
				Freshwater drum			4	
June 10	22.7	Bedrock	15	Emerald shiner	2.0	15	707	117
June 17	22.7	Bedrock		Postlarval	Postlarval fish	50	?	30
				Rainbow smelt	too small to			
				Gizzard shad	accurately count			
				Yellow perch				
				Adult				
				Rock bass			1	
				Yellow perch			3	
				Freshwater drum			50	
June 20	30.26	Bedrock	9	Rainbow smelt		?	1	?
				White bass			1	
				Yellow perch			8	
				Freshwater drum			2	
July 22	24.97	Rock & clay	5	Emerald shiner	2.0	80	40 212	18
				Yellow perch			2	
Sept. 9	45.4	Bedrock		Emerald shiner	0.5	100	15 708	17
				Rock bass			1	
				Freshwater drum			1	
Sept. 26	45.4	Bedrock	3	Emerald shiner	1.0	80	20 106	18
				Freshwater drum			1	
Sept. 27	45.4	Bedrock	5	Alewife	4.5		2 819	17
				Emerald shiner	0.2	100	6 383	
Oct. 2	37.8	Bedrock	7	Emerald shiner	0.75	120	33 134	13

TABLE 3. Summary of subsurface mortality estimates resulting from 37.33 kg/charge detonations in Long Point Bay, Lake Erie, October 2, 1975. Sampling was initiated 5 min after the blast and completed in 20 min.

Direction and no. of tows	Tow duration (min)	Tow depth (m)	Volume sampled (m^3)	Fish density per m^3	Other parameters	
E-W (2)	4.0	7.0	174	0.213	Fatal radius	120 m
W-E (2)	3.5	2.5–7.0	140	0.235	Mean depth	8 m
S-N (2)	4.0	2.5–7.0	152	0.291	Volume affected	361 912 m^3

	Estimated totals					
	Upper stratum		Lower stratum		Totals	
Species	No. killed	% mortality	No. killed	% mortality	No. killed	% mortality
Alosa pseudoharengus	633	78			633	77.8
Osmerus mordax	1 266	100	0	0	1 266	77.8
Notropis atherinoides	19 156	53	9 150	49	28 305	51.6

net avoidance. Acara and Smith (1971) tested the catching efficiency of rectangular nets in a clear stream and found that as long as the ratio of the surface area of the net mouth to bag was kept at 1:8 the catching efficiency would be close to 100%. Our conical nets fell just below the 1 to 8 ratio.

Investigations of fish losses after subsequent blasts off Tobermory, Ontario showed that the highest mortality occurred after the first detonation (Ontario Ministry of Transportation and Communications 1974). During the first monitored detonation (June 9) we observed up to 9 fish $\cdot m^{-2}$ while some 4 mo later, during the last monitored blast, the density of dead and injured fish remained ≤ 0.75 fish $\cdot m^{-2}$. The constant construction activity in the study area waters may have aided in reducing the overall mortality by frightening away the fish attempting to recolonize the area. Hubbs and Rechnitzer (1952) and Puke (1948) reported that fish recolonized the blast zone soon after a detonation. Brown and Smith (1972) observed schools of fish feeding in a blast zone within 1 d of a large detonation.

During 1976 the emerald shiner (species with highest losses in 1975) population (as determined by shoreline seining) declined slightly relative to 1975 numbers, a drop well within their normal range (Scott and Crossman 1973; Teleki 1975). The freshwater drum population was more noticeably affected; we recorded a significant drop in 1975 catch from offshore trap nets, relative to 1974 numbers caught. Catch of other common species remained unchanged indicating that the surface mortality counts were truly representative of the species composition and density of the adult fishes killed by the blasts.

Charges not buried in the bottom are associated with large lethal radii. Ferguson (1962) using 0.5 kg of high explosive reported fish kills 60 m from the blast. In another Lake Erie blasting operation, we estimated that 9-kg charges of a very high explosive would generate a lethal radius greater than 122 m.

In general construction blasting had little effect on the fishes, aside from destroying those within the lethal radius. The confined nature and timing of the detonation aided in reducing the overall impact, albeit the larval and postlarval fish mortalities were not assessed. Three physical and three biological variables emerged as the principal components in any blasting impact; the physical components were detonation velocity, density of material to be blasted and charge weight. Biologically important variables were fish shape, location of fish in the water column (Tyler 1960 cited by Falk and Lawrence 1973) and swimbladder development.

With an ever increasing use of explosives for both submarine construction work and resource exploration (Falk and Lawrence 1973), selected blasting operations should be monitored to provide a range of pressure data for various types of explosives at the same charge weight and collar depth, establishing minimum lethal pressures for different explosives. The f_2 function could then be determined for each condition. With these data, peak pressures for various charge sizes and distances could be estimated and a lethal radius, based on known MLP, predicted.

The indiscriminate placement of large charges in sensitive areas, e.g. river mouths during fish migration or spawning periods, could inflict significant damage on a fish population because regulatory agencies, for lack of assessment data, could not effectively prescribe the proper blasting guidelines. The information we presented should form the basis for the preparation of such guidelines.

Acknowledgments

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Two dedicated students, M. Ross and L. Dunn, aided in the often tedious task of field data collection.

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Fecundity of Witch Flounder (*Glyptocephalus cynoglossus*) from St. Pierre Bank and the Grand Bank of Newfoundland

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BOWERING, W. R. 1978. Fecundity of witch flounder (*Glyptocephalus cynoglossus*) from St. Pierre Bank and the Grand Bank of Newfoundland. J. Fish. Res. Board Can. 35: 1199–1206.

The fecundity of witch flounder from the Grand Bank and St. Pierre Bank was correlated to length, age and weight. In all cases a log–log fit best described the relationship between fecundity and all three variables. However, variations in fecundity were best described in terms of size rather than age. Significant variations in fecundity occurred between the three areas discussed. Significant differences between samples from different years occurred for the Grand Bank areas but not for St. Pierre Bank. A comparison of witch flounder fecundity for the East and West Atlantic indicated that the rate of egg production is generally the same in both areas but the number of eggs produced at each length group in East Atlantic witch flounder is higher than those in West Atlantic.

Key words: witch flounder, fecundity, length, age, weight

BOWERING, W. R. 1978. Fecundity of witch flounder (*Glyptocephalus cynoglossus*) from St. Pierre Bank and the Grand Bank of Newfoundland. J. Fish. Res. Board Can. 35: 1199–1206.

Nous établissons la corrélation entre la fécondité et la longueur, l'âge et le poids chez la plie grise du Grand banc et du banc Saint-Pierre. Dans tous les cas, un graphe log–log est celui qui décrit le mieux la relation entre la fécondité et ces trois variables. Cependant, les variations de fécondité s'expriment mieux en fonction de la taille que de l'âge. Il existe, entre les trois régions étudiées, de nettes variations de fécondité. On observe des différences significatives à différentes années dans les régions du Grand banc mais non sur le banc Saint-Pierre. Quand on compare la fécondité de la plie grise dans l'Atlantique oriental et occidental, on constate que le taux de production d'oeufs est généralement le même dans les deux régions, mais que le nombre d'oeufs produits par chaque groupe de longueur en Atlantique oriental est plus élevé qu'en Atlantique occidental.

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INFORMATION on the biology of the witch flounder (*Glyptocephalus cynoglossus*) in the Northwest Atlantic is quite limited. Evseenko and Nevinsky (1973) did a comprehensive study of breeding and development of witch flounder in the whole Northwest Atlantic and Bowering (1976a) published a detailed study on the distribution, age and growth, and sexual maturity of this species for the Newfoundland area. Although the fishery for this species dates back into the 1940s, it is only in recent years that it has become one of the major food fishes of the Northwest Atlantic. The annual landings in the Newfoundland–Labrador area have now reached about 30 000 t and all stocks are presently under international regulation.

The only previous work on the fecundity of witch flounder was done by Fulton (1891) who gives the results of investigations made on three specimens taken east of the Isle of Man, and Bagenal (1963) who did a more comprehensive study on 120 specimens taken from the Firth of Clyde in Western Scotland. The present material allows an area of comparison of witch fecundity from the northern and southern Grand Bank and St. Pierre Bank in relation to length, age, and weight (Fig. 1). The fecundity–length relationship for the total area is also compared with that of Bagenal (1963) for the Firth of Clyde.

Materials and Methods

COLLECTION OF OVARIES

All ovaries were collected during April–May 1974–77, late enough in the maturity cycle to enable separation of present generation ova from second generation ova. Ovaries which contained clear ova were rejected since it is probable that some of such ova may have already been extruded. All material was collected at sea during regular random stratified groundfish surveys by the Canadian research ship *A. T. Cameron* (Table 1).

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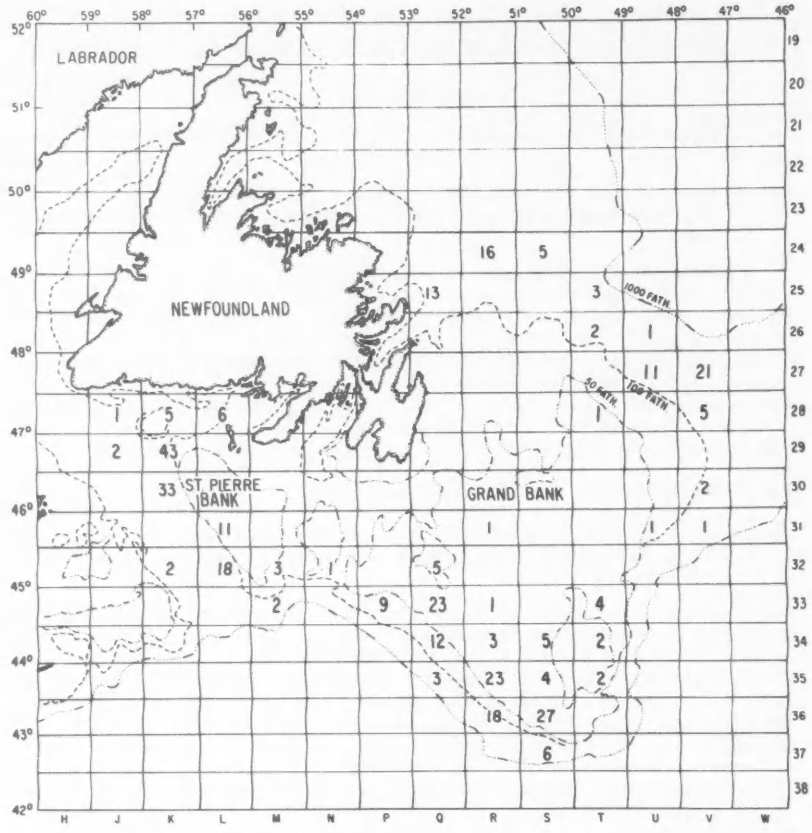

FIG. 1. Positions and sizes of fecundity collections in relation to ICNAF statistical division (grid on bottom and right axes).

TABLE 1. List of samples of witch flounder from which fecundities were estimated.

	ICNAF Div.	Year	No. of fish
	3L	1974	6
	3L	1975	11
	3L	1976	26
	3L	1977	37
Total	3L	1974–77	80
	3Ps	1974	23
	3Ps	1975	29
	3Ps	1976	35
	3Ps	1977	40
Total	3Ps	1974–77	127
	3NØ	1975	20
	3NØ	1976	101
	3NØ	1977	30
TOTAL	3NØ	1975–77	151
Grand total	3LNØ, 3Ps	1974–77	358

Otoliths, fork lengths to the nearest centimetre and whole weights (grams) were obtained from all fish sampled for ovaries. The ovaries, when extracted, were cut longitudinally and placed in jars of Gilson's fluid (Simpson 1951) which causes the breakdown of connective tissue after several weeks without damage to the ova. When the connective tissue was sufficiently broken down, the contents of the jars were washed through a series of fine screens of varying mesh size until the present generation ova could be completely separated from all other material. After cleaning, the ova were stored in a solution of 2-ethoxyethanol for several days before counting. This solution allows the ova to harden and prevents clumping by breaking down any excessive greasy or fatty material.

COUNTING

The ova were counted by means of the whirling vessel, a plankton subsampling device designed by E. Lea and G. Rollefsen of the Fisheries Directorate, Bergen, Norway, and later modified by Pitt (1965) for fecundity studies on American plaice. Subsampling and counting were carried out in the same manner as that described by Pitt (1964) for American plaice.

For analysis, the data were organized into three different

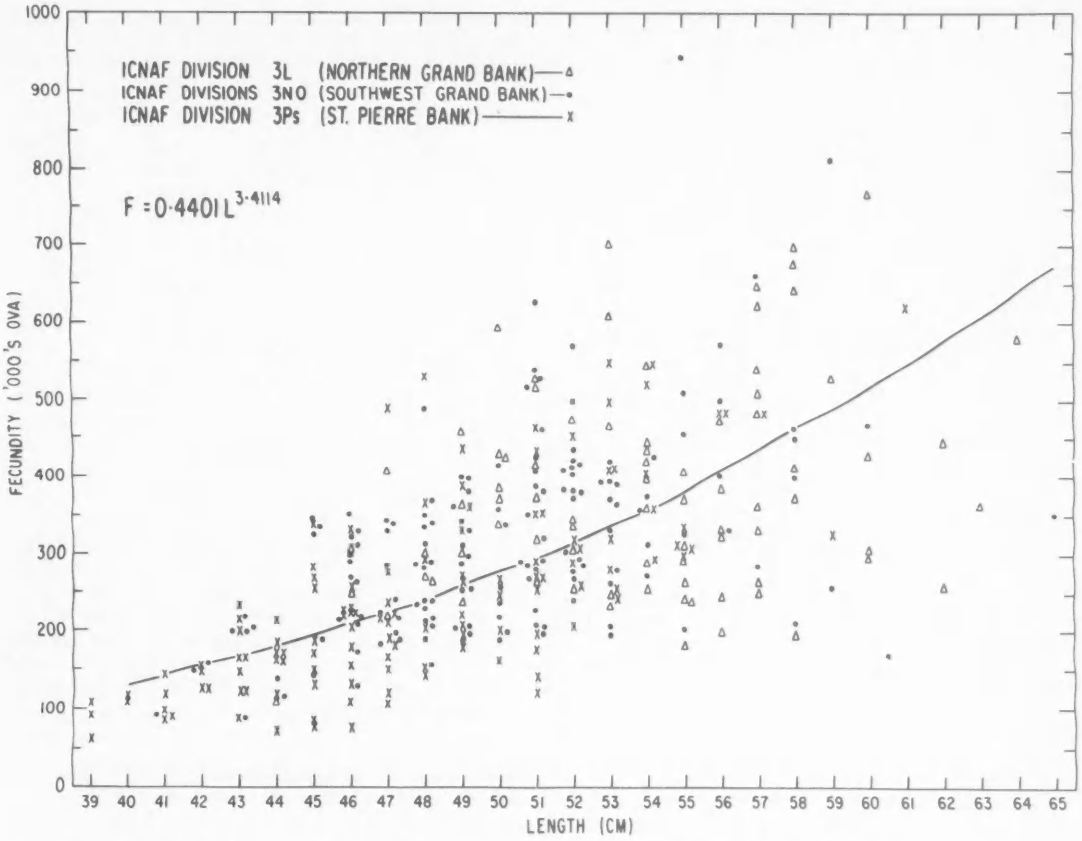

FIG. 2. Scatter diagram of witch flounder fecundity plotted against length (all data from all areas combined).

groups: (1) Northern Grand Bank (ICNAF Division 3L); (2) Southwest Grand Bank (ICNAF Division 3Ø and part of 3N); (3) St. Pierre Bank (ICNAF Division 3Ps). According to the distribution charts presented by Bowering (1976a), these three areas have separate concentrations of witch flounder.

Results

In relating fecundity of witch flounder to length, age, and weight, four types of mathematical plots (arithmetic–arithmetic, arithmetic–log, log–arithmetic, and log–log) were performed. In all cases the log–log plot gave the highest linear correlations.

FECUNDITY VS. FISH LENGTH

Fecundity (F) increased with length (L) (Fig. 2) according to the following relationship:

$$\text{Log } F = 3.4114 \text{ Log } L - 0.3565$$

(where F = fecundity, and
L = fork length in centimetres)

The correlation coefficient (r) for this relationship is significant (Table 2).

Log–log straight lines were also fitted for the three areas independently (Fig. 3A); all correlation coefficients were significantly different from zero at the 1% significance level (Table 2). Analyses of covariance (Zar 1974) showed that there were significant differences in both the slopes (the rate of egg production) and the adjusted means (quantity of eggs produced) of the three lines (Table 3, Test 1; Fig. 3A). Further analyses of covariance were carried out on 3L–3NØ, 3L–3Ps, and 3NØ–3Ps to determine if the variance was due to the inclusion of one particular area (Table 3, Tests 2, 3, and 4). However, except for the adjusted means in the 3L–3NØ test, all other variables were significantly different indicating that all areas contributed something to the total variation.

Analyses of covariance were also carried out to determine if there were annual differences for the three areas (Table 3; Fig. 4). For the 2 yr compared for the northern Grand Bank (3L), there was no difference in the rate of egg production between years but there

TABLE 2. Regression constants and tests for significance of correlations of fecundity with length and age for witch flounder; M = slope, and log K = intercept of fitted log–log straight lines.

	ICNAF Div.	No. of fish	M	Log K	r	t	P
Length	3L	79	1.423	3.094	0.306	2.820	<0.01
	3NØ	150	2.877	0.381	0.584	8.662	<0.01
	3Ps	127	4.084	−1.526	0.746	12.524	<0.01
	All areas	356	3.411	−0.356	0.683	17.643	<0.01
Age	3L	79	0.092	5.448	0.044	0.387	0.70
	3NØ	147	0.592	4.775	0.243	3.017	<0.01
	3Ps	125	1.342	3.772	0.555	7.400	<0.01
	All areas	351	0.956	4.320	0.367	7.392	<0.01
Weight	All areas	357	0.9313	2.6982	0.6772	17.340	<0.01

TABLE 3. Summary of results of covariance analyses for regressions of fecundity against length and age for witch flounder.

		Mean squares				Mean squares			
	Areas tested	Within samples	Regression coefficients	F	P	Common regression	Adjusted means	F	P
Length	1) 3L, 3NØ, 3Ps, 1974–77	0.0212	0.2367	11.16	<0.001	0.0224	0.1952	8.71	<0.001
	2) 3L–3NØ, 1974–77	0.0206	0.1287	6.24	0.013	0.0211	0.0045	0.21	0.651
	3) 3L–3Ps, 1974–77	0.0228	0.4467	19.58	<0.001	0.0249	0.0995	4.00	0.044
	4) 3NØ–3Ps, 1974–77	0.0209	0.1353	6.47	0.011	0.0213	0.2893	13.56	<0.001
	5) 3L, 1976, 1977	0.0184	0.0055	0.27	0.846	0.0179	0.1490	8.32	<0.001
	6) 3NØ, 1976, 1977	0.0161	0.1398	8.69	<0.001	0.0178	0.1203	6.77	0.002
	7) 3Ps, 1974, 1975, 1976, 1977	0.0219	0.0193	0.88	0.545	0.0218	0.0522	2.39	0.071
Age	8) 3L, 3NØ, 3Ps, 1974–77	0.0297	0.2831	9.53	<0.001	0.0311	1.1990	38.50	<0.001

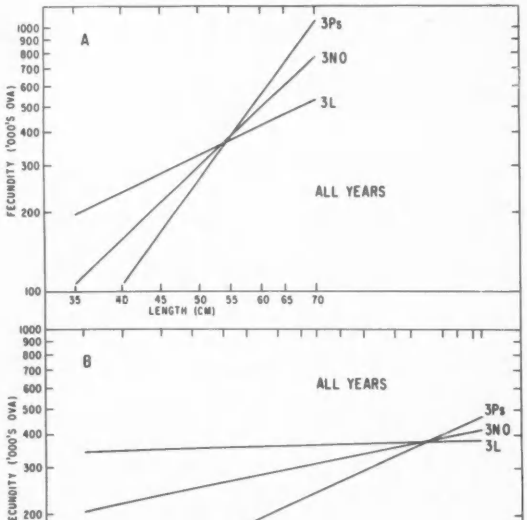

FIG. 3. Log–log regression of witch flounder fecundity on length and age for ICNAF Division 3L, 3NØ, and 3Ps.

was a significant difference in the number of eggs produced. For the 2 yr compared for the southern Grand Bank both the rate of egg production and the number of eggs produced were significantly different for each year. The rate of egg production and the number of eggs produced were the same for each of the 4 yr compared for St. Pierre Bank (3 Ps). These results indicate that annual fluctuations in the rate of egg production and number of eggs produced may or may not occur.

FECUNDITY VS. FISH AGE

The straight line equation for fecundity plotted against age is:

$$\text{Log } F = 0.9564 \text{ Log } A + 4.3202$$

Variation (Fig. 5) was much greater and the correlation coefficients lower for each area than for plots of fecundity against length (Table 2). However, all correlation coefficients except northern Grand Bank (3L) were significantly different from zero. Analysis of covariance between areas (Table 3, Test 8) indicated that differences exist both in the elevation of the lines (rate of egg production) and vertical separation of the lines (number of eggs produced) (Fig. 3B).

The growth curves presented in Fig. 6 indicate that the growth rates of the northern and southern Grand Bank fish are almost identical whereas the St. Pierre

Bank fish are substantially slower growers. Thus it appeared that the total Grand Bank area could be combined. However, an analysis of covariance (Table 3, Test 2) showed that the slopes of the length–fecundity plots differed; therefore, the two areas were kept separate.

Fecundity vs. Length and Age

On the basis of the correlation coefficients presented in Table 2, it is obvious that fecundity is related to both length and age. Considering the higher correlation coefficients and the lesser variation (Fig. 2 and 5) fecundity is more dependent on length than age. However, since length is a function of age, a multiple correlation analysis was applied to each area separately and then to all areas combined (Table 4), to determine the contribution of length and age to fecundity variations.

For all areas the coefficient of determination is greater for equations using length only as compared to using age only. When both length and age are used together, the coefficient of multiple determination was also slightly higher than if length had been used alone for all areas tested. The increase in the amount of explained variation, however, was not significant in either of the tests performed when the coefficient of partial determination ($r_{13.2}$) was employed.

For ages 14 and 15 from St. Pierre Bank, plots of fecundity against length (Fig. 7) illustrate that the variation in fecundity is explained by length variation even within the same age-group thus explaining the wide scatter of points for the fecundity–age plot in Fig. 5. From this, and the multiple correlation analysis, one may conclude that, for all practical purposes, fecundity may be adequately explained in terms of length alone.

Fecundity vs. Weight

Only whole weights were available to plot against fecundity which may cause slight variations in fish weight at the same length. However, considering the size of the viscera and the amount of food a witch flounder may consume, the variation would be very small (unpublished data of the St. John's Biological Station). Fecundity plotted against weight (Fig. 8) gave the following linear relationship:

$$F = 252.06W + 70892.04$$

The correlation coefficient (r) for this relationship is 0.595 and is statistically significant ($P < 0.01$). A good linear relationship is not surprising in this case since the length–weight relationship for this species is $W = 0.00063709L^{3.6142}$ (Bowering 1976b) and the fecundity length relationship is $F = 0.4401L^{3.4114}$ both of which are similar.

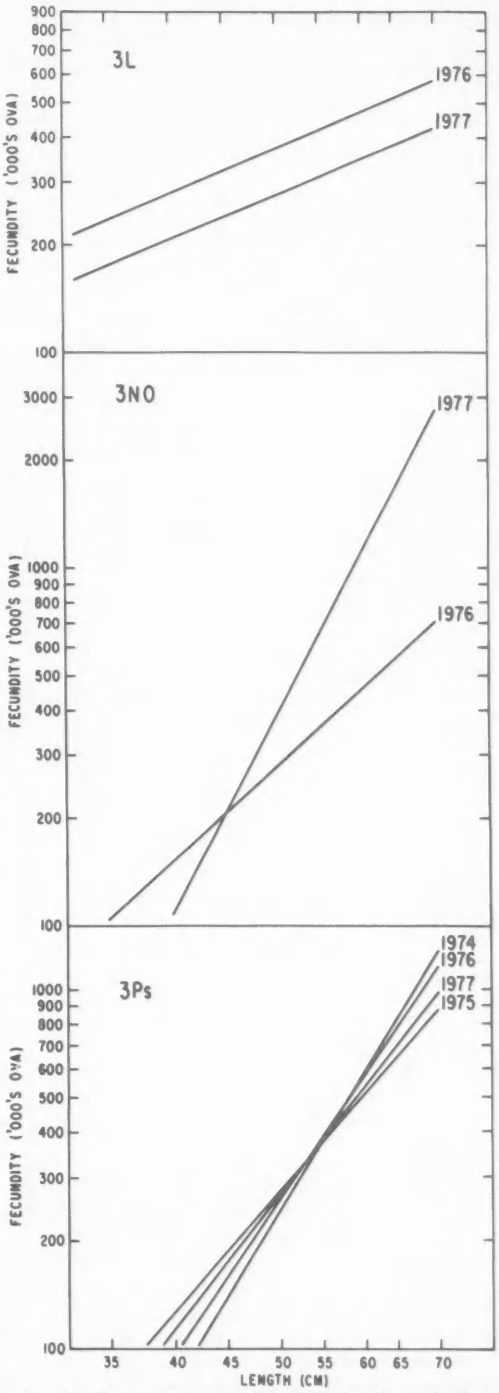

FIG. 4. Comparison of witch flounder fecundity between years for ICNAF Divisions 3L, 3NØ, and 3Ps.

TABLE 4. Summary of statistics for multiple correlation of fecundity, age and length of witch flounder. X_1–Log fecundity, X_2–Log length, and X_3–Log age. Estimating equation $X_1 = a_{1.23} + b_{12.3}X_2 + b_{13.2}X_3$.

		ICNAF Div,			All areas
		3L	3NO	3Ps	
No. of fish	N	79	150	127	356
Coefficient of determination for X_1 and X_2	r^2_{12}	0.094	0.341	0.549	0.461
Correlation coefficient for X_1 and X_2	r_{12}	0.306	0.584	0.741	0.679
Coefficient of determination for X_1 and X_3	r^2_{13}	0.002	0.059	0.308	0.135
Correlation coefficient for X_1 and X_3	r_{13}	0.043	0.243	0.555	0.367
Coefficient of multiple determination	$R^2_{1.23}$	0.118	0.348	0.557	0.462
Coefficient of multiple correlation	$R_{1.23}$	0.344	0.590	0.746	0.680
Coefficient partial determination	$r^2_{13.2}$	0.0265	0.0106	0.0181	0.0019
	t for $r^2_{13.2}$	1.4478	1.6517	1.5180	0.8209
	P for $r^2_{13.2}$	0.10	0.10	0.10	0.20–0.25

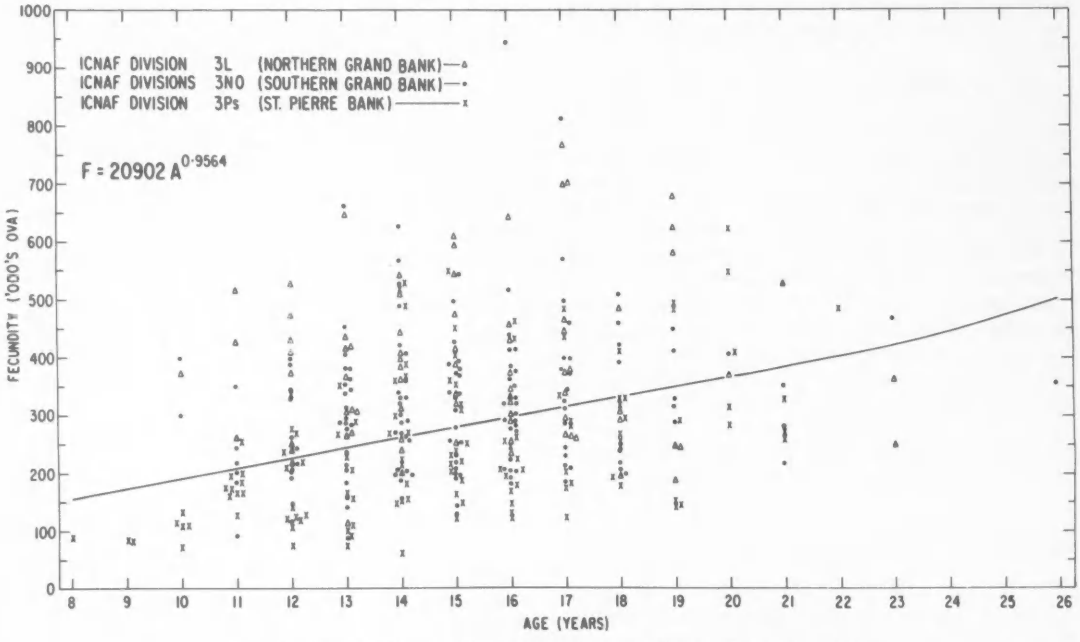

FIG. 5. Scatter diagram of witch flounder fecundity plotted against age.

Discussion

In studying the fecundity of the North Sea plaice (*Pleuronectes platessa*), Simpson (1951) expressed the relationship between fecundity and length by the "cube law" $F = KL^3$. Other investigators such as Begenal (1963), Pitt (1964), and May (1967), in studying fecundity of other species, used this relationship and found that, more often than not, the exponent of the equation fell between 3 and 4. This was true for this study for all areas combined; however, differences did exist from one area to another.

The exponent in the age–fecundity relationship is rather low (0.95) in comparison to most other species (2.00). However, the life span of witch flounder is very long and a range of length groups are required to make up a single age-group; in this particular case the average fecundity at each age is close to a straight line relationship on an arithmetic plot (Fig. 5) which would partially explain the low exponent value.

Comparison of correlation coefficients (Table 2) shows that fecundity can be explained in terms of weight almost as well as by length. May (1967) suggests that, for Atlantic cod (*Gadus morhua*), weight is variable through seasonal feeding habits and this variability can provide a bias in the data if they are not collected at the same time. This could also be true of this species but to a much lesser extent than for cod considering the volume of the stomach in relation to body size. In any case, the length is much less variable

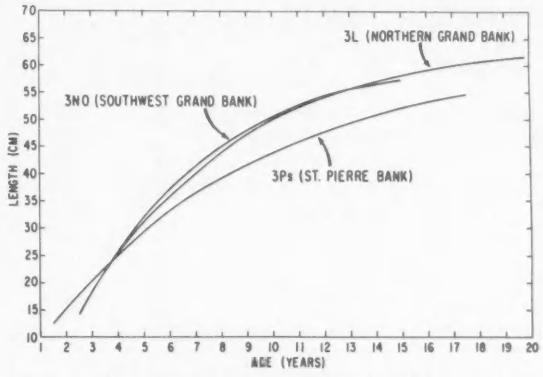

FIG. 6. Growth curves for female witch flounder from ICNAF Divisions 3L, 3NØ, and 3Ps.

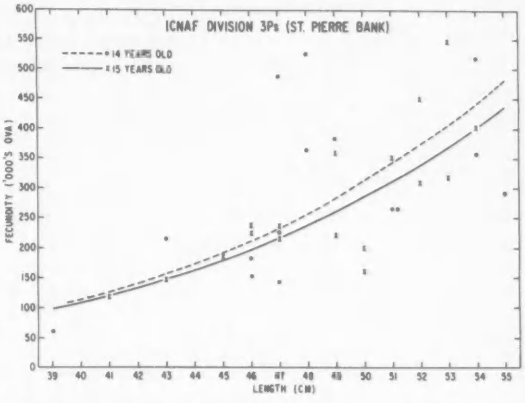

FIG. 7. Regressions of witch flounder fecundity on length for fish of ages 14 and 15 from St. Pierre Bank (ICNAF Division 3Ps).

and is much easier to measure than weight, especially when done at sea.

The differences in the fecundity for the three areas may be explained partly in terms of sexual maturity and spawning. Hodder (1963) suggested that for haddock (*Melanogrammus aeglefinus*) the more often a fish has spawned, the more fecund it is. Because of difficulty in distinguishing spawning zones from witch flounder otoliths, it was difficult to investigate this possibility to any great extent. However, Bowering (1976a) showed that witch flounder from St. Pierre Bank reached sexual maturity at an older age than from the other two areas. One could therefore assume that, at comparable ages, St. Pierre Bank witch flounder would have spawned fewer times than those of the other areas. Figure 3B shows that St. Pierre Bank fish are less fecund at comparable ages until very old (22 yr) as compared to the other areas, indicating that this possibility does exist, ie. that the more times a fish has spawned the more fecund it is.

Annual variations in fish fecundity were first considered seriously by Simpson (1951) who worked on plaice (*Pleuronectes platessa*). He provided estimates of numbers in the population by means of planktonic

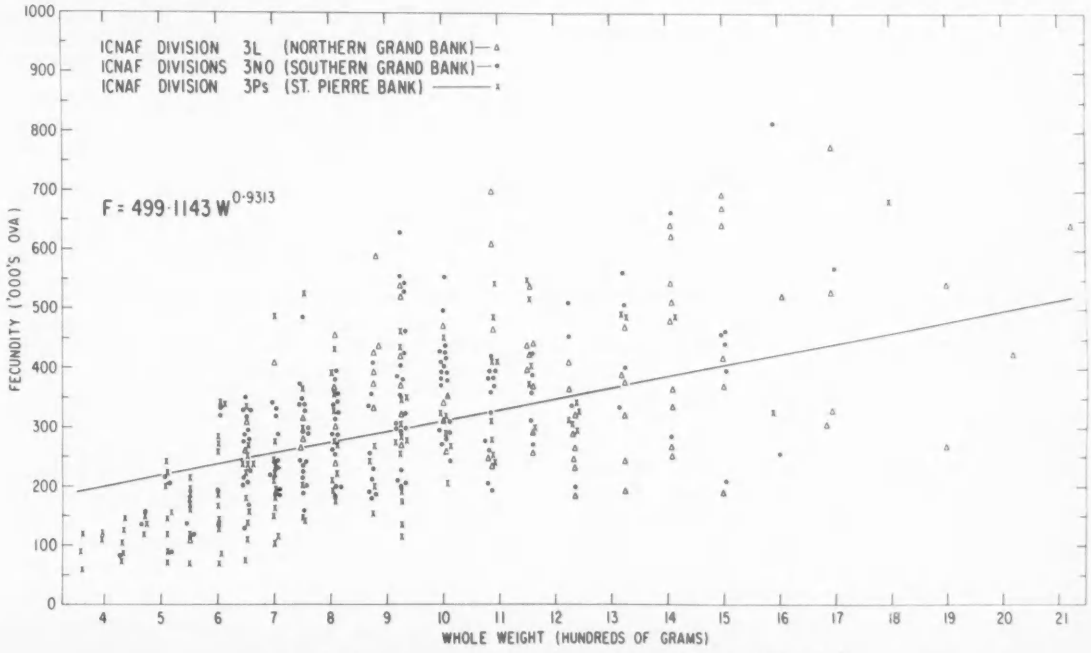
ICNAF DIVISION 3L (NORTHERN GRAND BANK)
ICNAF DIVISIONS 3NO (SOUTHERN GRAND BANK)
ICNAF DIVISION 3Ps (ST. PIERRE BANK)
F = 499·1143 W^0·9313
FECUNDITY ('000'S OVA)
WHOLE WEIGHT (HUNDREDS OF GRAMS)

FIG. 8. Scatter diagram of witch flounder fecundity on whole weight.

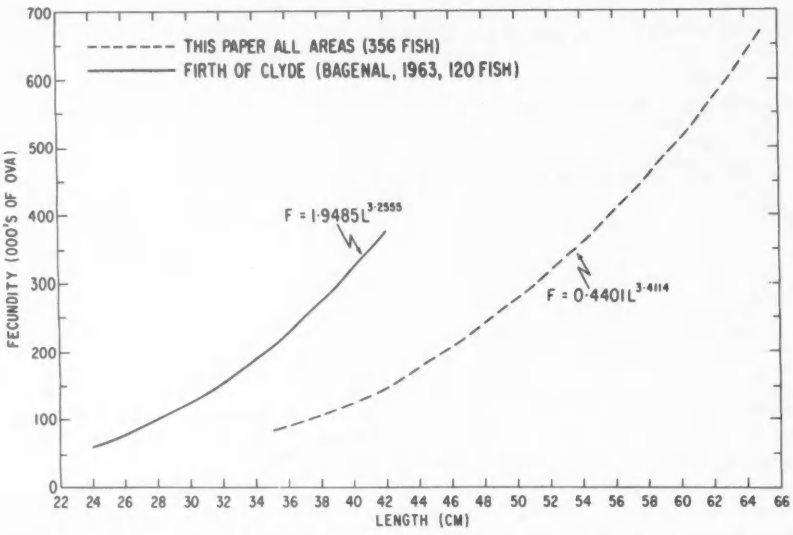

FIG. 9. Comparison of fecundity–length regressions for West Atlantic and East Atlantic witch flounder.

egg surveys, and found that fluctuations occurred annually. Bagenal (1957) also found significant differences in the fecundity of American plaice (*Hippoglossoides platessoides*) for the years 1954–56 in European waters and Pitt (1964) found annual fluctuations in the fecundity of this same species in Canadian waters. A variety of possible explanations has been offered for these differences; however, few have been based on any real evidence. Hodder (1963) related annual fluctuations in haddock fecundity with fluctuations in air and water temperatures. He found that years of unusually low temperature were followed by low fish fecundity 2 yr later and vice-versa. He suggested that the years of low temperatures cause crowding which resulted in less food and less growth and probably caused less fecundity 2 yr later, the opposite being the case for years of high temperature. With a very slow-growing long-lived species such as witch flounder, however, it is very difficult to detect any annual differences in the rate of growth.

It is interesting to compare the length–fecundity relationship of the Grand Bank–St. Pierre witch flounder and that of Bagenal (1963) for the Firth of Clyde in western Scotland (Fig. 9). The exponents of the equations are very similar, meaning that the rate of egg production is similar. However, the position of the curves differs because the European form of this species does not grow to the same maximum size as the Northwest Atlantic form and matures as a much smaller size.

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Zooplankton Sample Variation on the Scotian Shelf[1]

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SAMEOTO, D. D. 1978. Zooplankton sample variation on the Scotian Shelf. J. Fish. Res. Board Can. 35: 1207–1222.

Zooplankton sampling experiments on the Scotian Shelf during September 1973 and August 1974 using vertical tows demonstrated that numbers of many zooplankton species had a periodic fluctuation over 26 h. The fluctuations appeared related to the tide during 1974 but not in 1973. The periodic fluctuations accounted for a major portion of the sample variation in many species during both years. The mean numbers of small forms of zooplankton (copepods) obtained from the 26-h time series on a single station were very similar to means obtained during the same cruise over a wide area of the shelf. This suggested that a single station sampled over a period of two tidal cycles may be representative of the sample variation encountered over a wide geographic area of many hundreds of kilometres. Only *Calanus* and *Pseudocalanus* were correlated during all the experiments and from year to year. The abundance and distribution of the other species of zooplankton compared from one year to the next were not significantly correlated. This suggested the species populations were independent of one another.

Key words: zooplankton, sample variation, time series, Scotian Shelf, fish larvae, Copepoda, tide

SAMEOTO, D. D. 1978. Zooplankton sample variation on the Scotian Shelf. J. Fish. Res. Board Can. 35: 1207–1222.

Des prises verticales de zooplancton effectuées sur le plateau néo-écossais en septembre 1973 et en août 1974 démontrent que plusieurs zooplanctontes accusent des fluctuations périodiques en 26 h. Les fluctuations semblent liées à la marée en 1974 mais non en 1973. Pendant les deux années, les fluctuations périodiques expliquent une grande partie de la variation dans les échantillons. Le nombre moyen de petits zooplanctontes (copépodes) recueillis au cours des périodes de 26 h à une station donnée ressemble beaucoup aux moyennes observées durant la même croisière sur une superficie étendue du plateau. Cela donne à croire que l'échantillonnage à une station unique sur une période de deux cycles de marée peut être représentatif de la variation observée dans les échantillons sur une grande étendue de plusieurs centaines de kilomètres.

Il y a corrélation durant toutes les expériences et d'une année à l'autre seulement entre *Calanus* et *Pseudocalanus*. Pour ce qui est de l'abondance et de la distribution des autres espèces de zooplancton, il n'y a pas de corrélation significative d'une année à l'autre. Les populations des diverses espèces seraient donc indépendantes les unes des autres.

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AN understanding of zooplankton distribution and concentrations in the marine environment has been hindered by many factors. These range from limitations in plankton net design and technology which result in problems of net avoidance (reviewed by Clutter and Anraku (1968) and Laval (1974)) and loss of zooplankton through the mesh of the nets (Vannucci 1968; Bernhard et al. 1973) to lack of experimental designs that will detect different patch sizes and densities. In addition to these problems high sample variability has resulted in wide confidence limits on estimated mean values of zooplankton numbers, making comparisons between areas difficult.

Sameoto (1975) found in a nearshore environment that the numbers of many zooplankton species from a single station showed a periodic fluctuation similar to the tide when sampled hourly for 26 h. The periodicity accounted for 26–81% of the sample variance of the time series of different zooplankton species. The purpose of the present study was to determine whether similar periodicity in zooplankton numbers could be detected in fixed stations on the Scotian Shelf and, if so, to measure its effect on the total sample variance.

The means and variances of the single station data

[1]Bedford Institute of Oceanography Contribution.

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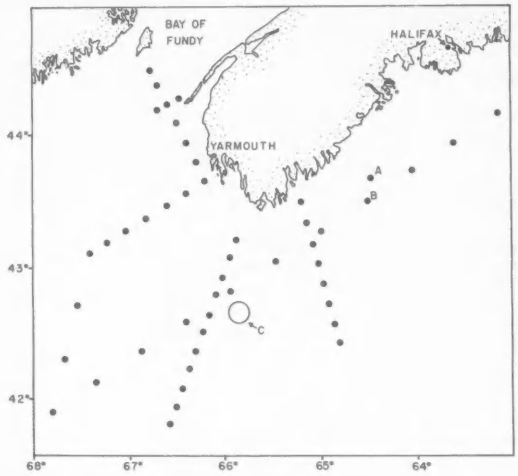

FIG. 1. Location of the sampling stations. A, 26-h station on Roseway Bank, 1973; B, 26-h station on Roseway Bank, 1974; C, area in which the random stations were located, dots are positions of the shelf stations.

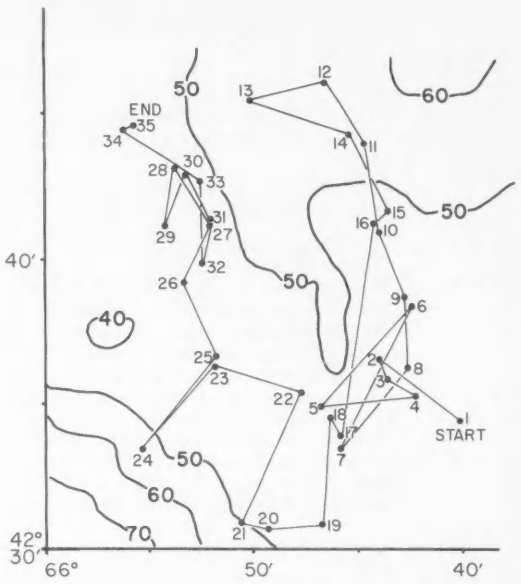

FIG. 2. Positions and sequence of the random stations. Depth contours are shown in metres.

series were compared with those from data series taken over an area of 200 km² and a data series taken from a sampling grid covering several hundred square kilometres on the Scotian Shelf. The aim of this comparison was to determine the degree to which the results from the different sampling schemes differed. These studies provided an excellent opportunity to look at zooplankton species associations and determine whether they were fortuitous or real.

Methods

Areas of Study

Geographically fixed sampling stations on the Scotian Shelf were sampled during September 20–21, 1973 and August 9–10, 1974 between 40 and 55 km from shore, respectively, in 64 m of water (Fig. 1). These stations were marked with anchored floating buoys to ensure that the ship remained exactly on station for the 26-h sampling period. A series of 47 sampling stations on 5 major transects were taken from August 8 to 15, 1974 on the southwest Scotian Shelf (Fig. 1).

On August 11, 1974, an area was selected off the southwest tip of Nova Scotia (Fig. 1) where samples were taken on a series of stations determined by selecting at random a ship's course and traveling at an average speed of about 5 knots for 30 min and stopping. A new random course was picked to each consecutive station, using the previous station as the starting point (Fig. 2). The random course selection was made by drawing a piece of paper marked with a degree bearing from a container that held papers with all 360 deg. The distance between the stations was not constant because of factors of wind, current, and tides.

Sampling Gear and Procedure

All samples other than those on the transects were from vertical tows taken from within 2 m of the bottom to the surface with a 0.5 m diam bongo net with Tsurumi-Seiki Kosakusho Co. flow meters in both sides. The frame of the net was painted black, and the nets were dyed a dark green to reduce animal avoidance of the nets. The monofilament mesh of the nets had a pore size of 243 μm. The nets were towed at a speed of 1.5 $m \cdot s^{-1}$ which was fast enough to prevent net avoidance by copepods (Laval 1974). Two tows were taken 4 min apart every 45 min on the marked stations for a period of 26 h. The same procedure was followed on the random stations except a new station was sampled every 45 min.

On the transect stations over the shelf single oblique tows were made with the bongo net from the bottom to the surface at a net speed of 1.5 $m \cdot s^{-1}$. Samples were taken during the day and night.

All samples were preserved in a 5% seawater and Formalin solution. Large zooplankters such as medusae, euphausiids, chaetognaths, and fish larvae were removed, identified, counted, and wet weighed. The remaining sample, consisting primarily of copepods, was vacuumed over a 30-μm filter until the water ceased to drip and then weighed for a wet biomass estimate.

The copepod samples were split a number of times and one of the portions used for species identification and counts. The reproducibility error of this counting method was about 5% (Sameoto 1975).

Statistical Methods

Biomass measurements and numbers of animals of each species found were converted to numbers 100 m^{-3} for large animals and cubic metres for copepod-sized animals using the flowmeter data. These numbers were transformed to $\log_{10}(x + 1)$ to normalize the distribution and decorrelate

the mean and variance. A hierarchical analysis of variance (Bennett and Franklin 1954) was then calculated for the data. The data were partitioned into three levels of sample components. The degrees of freedom were as follows:

Level	Source of estimate	df
1. Time (35 units of 45 min each)	Between times	35 -1
2. Tow number (2 tows in each time unit)	Between tows within time	([2-1] 35)
3. Sample number (2 samples in each tow	Between samples within tows and time	((2[2-1]) 35)

The mean values for the individual zooplankton components in samples for each tow were determined and plotted against time for each of the 26-h experiments. These mean values were used in a Fourier regression analysis with 1–5 harmonics to determine possible periodicity in the data over the 26-h period. For a description of the method see Dixon (1968) and Sameoto (1975).

Correlation coefficients (r) were calculated for all possible pairs of zooplankton components taken during each of the experiments to determine whether significant r values existed between different species, using the means of the four samples taken each 45-min time interval or in the case of the shelf stations, the values from each of the samples taken on each station. The data were first transformed to $\log_{10}(x + 1)$ before calculations.

Autocovariance analyses (Dixon 1968) were run on all the species in the two Roseway Bank experiments that had significant periodic regressions. This provided a measure of the wave length by multiplying the first wave crossing by 4, and a series of correlation coefficients at 0–15 time lags between all possible combinations of the species and the tide in each of the experiments.

The mean values given in the tables for the different species are the geometric means calculated from the $\log_{10}(x + 1)$ transformed data. The 95% confidence intervals are represented by the method given by Bagenal (1955).

TABLE 1. List of zooplankton components (numbers unless otherwise stated) with significantly different F values in the hierarchical analysis of variance for the three 26-h experiments. Experiment A, 1973 Roseway Bank; B, 1974 Roseway Bank; C, 1974 Random Station experiment. 1. Means numbers were significantly different at $P < .05$ between hours. 2. Numbers significantly different between tows within the hours. 0. No significant difference between numbers at any level. A blank space indicates there were no data.

	Experiment		
	A	B	C
Sagitta eggs	1		
Sagitta <5 mm			1
Sagitta	1	1	1
Satitta (biomass)	1, 2	2	1
Copepod (biomass)	1	1, 2	1
Calanus	1	1	1, 2
Pseudocalanus	1	1	1
Temora	1		1
Acartia	0	1	
Oithona	1		
Centropages	1	1	
Metridia	0		
Evadne	0	1	1
Spiratella		1	1
Clione larvae		0	1
Euphausiid eggs		1	1
Euphausiid larvae		1	1
Thysanoessa longicaudata		1	1
Crab larvae		1	0
Salps			1
Salps (volume)			1
Silver hake eggs		0	1, 2
Red hake eggs		0	1
Red hake larvae			2
Silver hake larvae			1, 2
Tomopteris		1	1
Medusae (volume)			0

Results

In all three 26-h experiments a majority of the zooplankton components were significantly different between hours (Table 1). However, the difference between tows within the hours was significant in only 7 cases out of 51. A significant difference between the tows within the hours meant that the difference in species numbers between tows taken 4–5 min apart was greater than the difference between the two samples taken during the same tow. This suggested that the distribution of these species was more heterogeneous than species that did not have a significant F value between the tows. The components that showed the largest variance between the tows were the larvae of red hake (*Urophycis chuss*) and silver hake (*Merluccius bilinearis*).

When significance was found between tows or insignificance found between times it was with a species that had a very low sample density, thereby giving a large sample to sample variance. In each case where there were significant differences between the hours as well as the tows the difference between the hours was larger indicating that these populations had greater heterogeneity on a time scale of hours than a scale of 4–5 min.

Paired t-tests showed no significant difference between samples taken during the same tow for any of the components.

ROSEWAY BANK 1973

Several species on the Roseway Bank station showed a significant periodic fluctuation ($P < .05$) with time (Fig. 3a and b). Generally it was the more abundant animals such as the copepods that showed a regularity in their fluctuation. Most species with a maximum density of $<100 \cdot m^{-3}$ appeared to have random fluctuations, based on the sampling frequency of this experi-

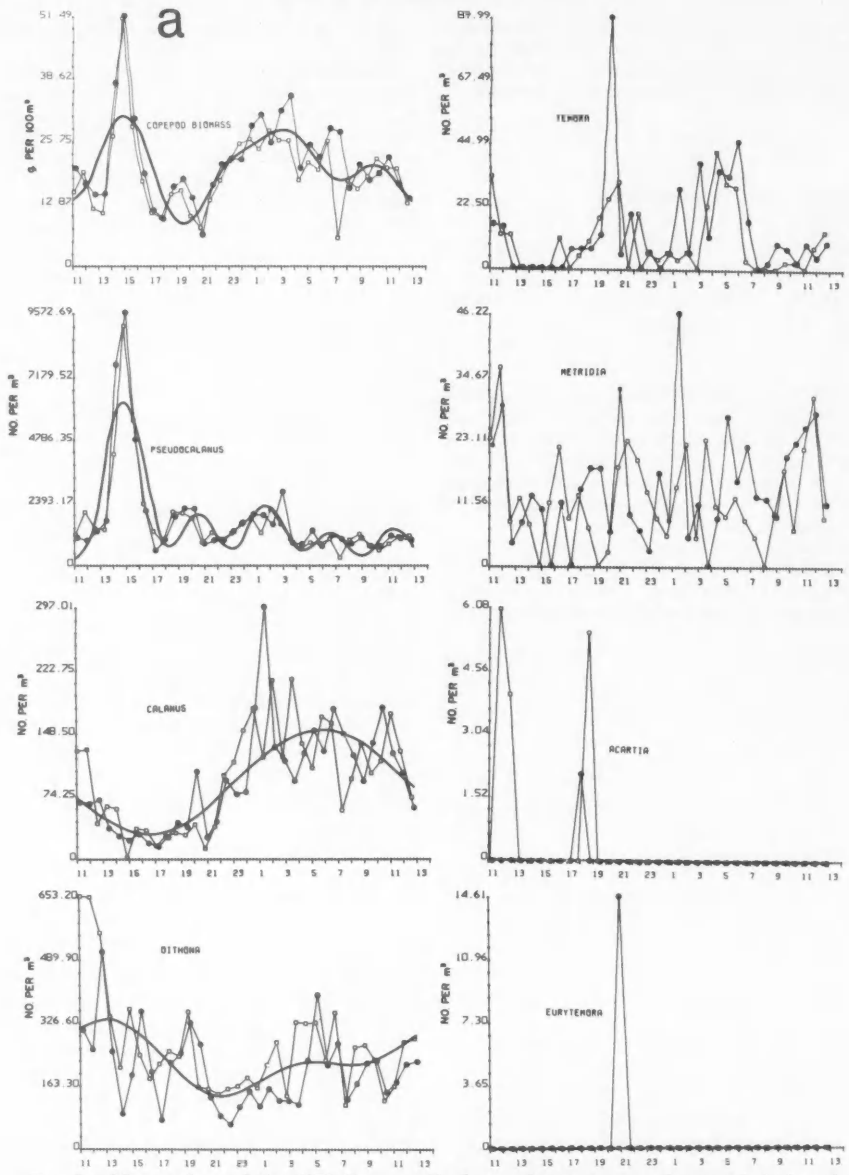

FIG. 3. Mean values of the 1973 Roseway Bank sample components for tow 1 (○) and tow 2 (●) and fitted periodic regression curves and the tidal fluctuation curve for 26 h.

ment. Copepod biomass was the only component whose fluctuations showed any similarity with the tide (Fig. 3a).

There was little similarity in the form of the fluctuations between the different copepod species (Fig. 3a). The copepod biomass changes with time were a reflection of the change in numbers per cubic metre of *Pseudocalanus* and *Calanus* (Fig. 3a) the two most abundant species. This was most evident in the first peak of the copepod biomass which was almost entirely due to *Pseudocalanus*. The fluctuations of these two species appeared to be unrelated. *Metridia* had what appeared to be a random fluctuation with time, whereas *Temora* numbers increased in three pulses, suggesting that concentrations of animals were passing by the sampling station (Fig. 3a). The increase in medusae

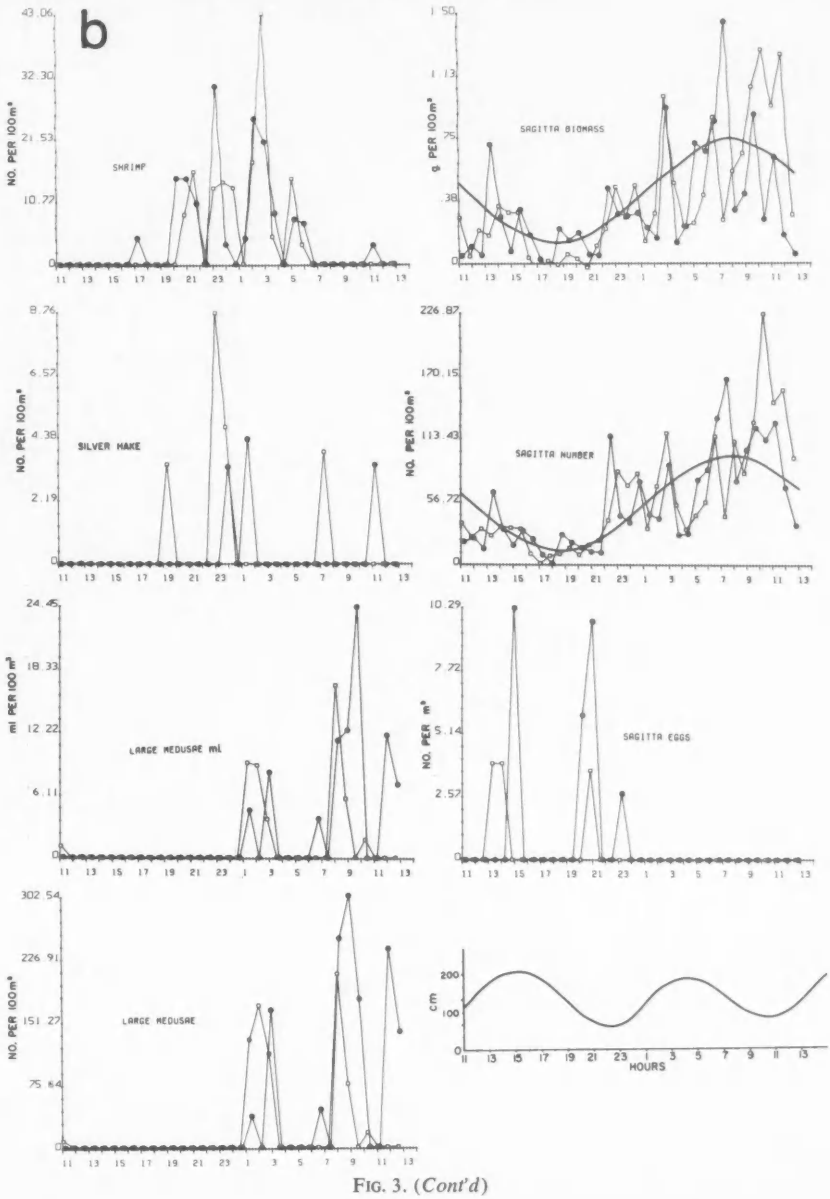

FIG. 3. (*Cont'd*)

during the latter half of the experiment indicated that a concentration of these animals moved past the station (Fig. 3b).

ROSEWAY BANK 1974

In the Roseway Bank experiment in 1974 more species showed periodic fluctuations than in 1973 (Fig. 4). The copepods, with the exception of *Temora* (Fig. 4a), appeared to fluctuate in phase with each other and 180° out of phase with the tide. *Temora* fluctuation appeared essentially random with no trend. The copepod taxa, except *Temora*, euphausiid larvae, *Spiratella*, and *Sagitta* all had similar fluctuation patterns (Fig. 4b), which indicates that water containing larger numbers of these species passed the sampling station during the two low tide periods (Fig. 4a).

The euphausiid eggs initially appeared in high num-

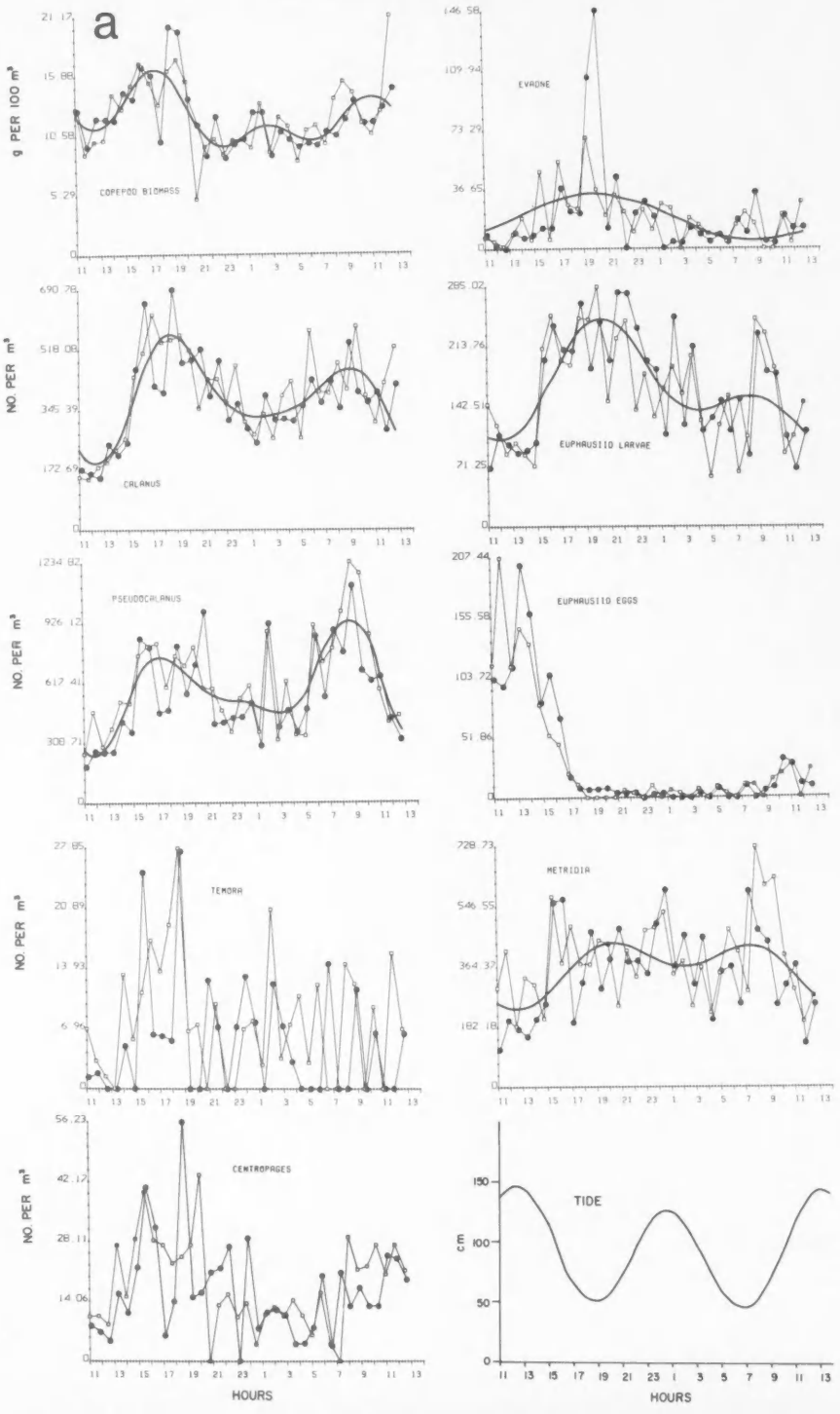

FIG. 4. Mean values for the 1974 Roseway Bank sample components. For explanation see Fig. 3.

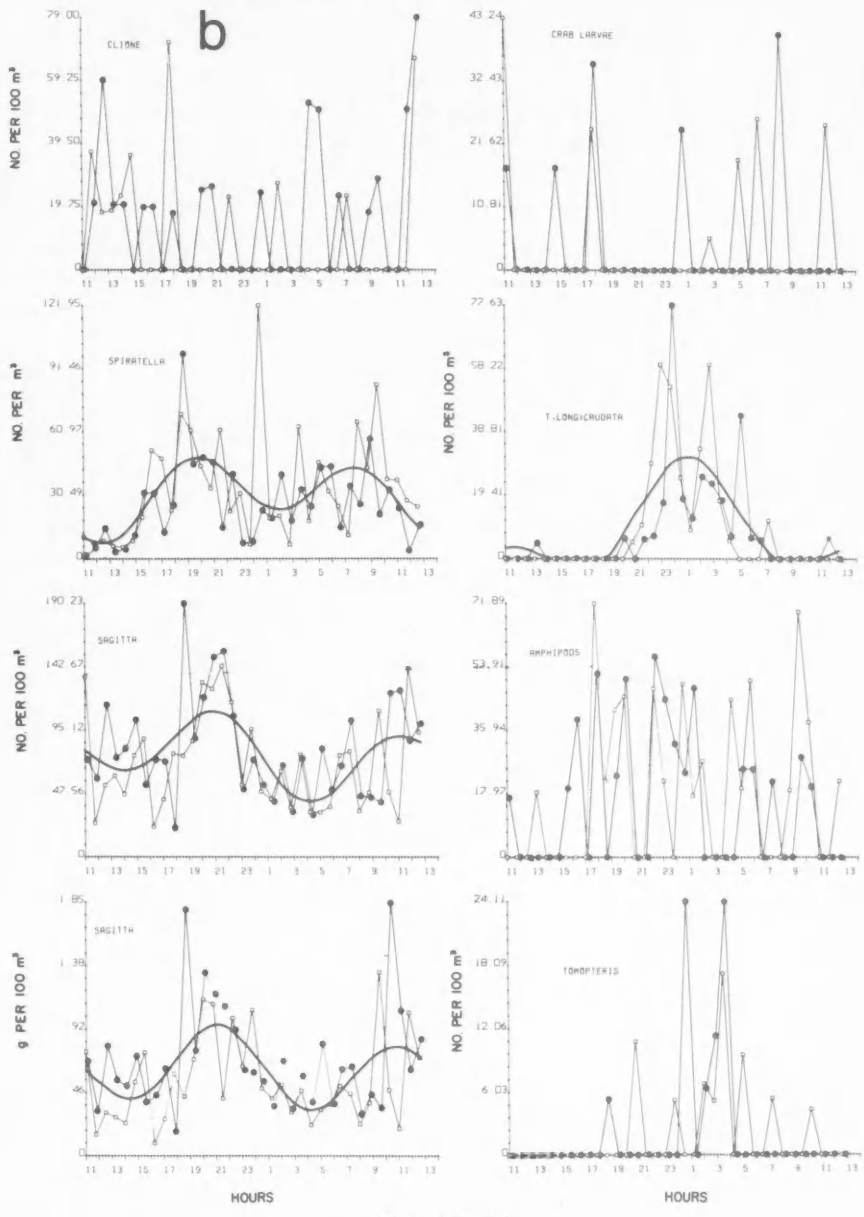

FIG. 4. (*Cont'd*)

bers but quickly dropped in density and remained low for the duration of the experiment (Fig. 4a), suggesting that a high concentration of eggs moved past the station and did not return during the 26-h period.

The euphausiid *Thysanoessa longicaudata* and the polychaete *Tomopteris* increased in abundance during the evening and night but decreased at dawn (Fig. 4b). This was probably due to the animals migrating vertically at dusk making them more susceptible to capture. The characteristic increase in the density of *Sagitta* at night seen during the 1973 experiment and by Sameoto (1975) was not found. This suggested that if there were a vertical migration at night it was masked by changes in the density of the animals as the water mass moved past the station.

The nets were 2 m long thus the mouth of the ver-

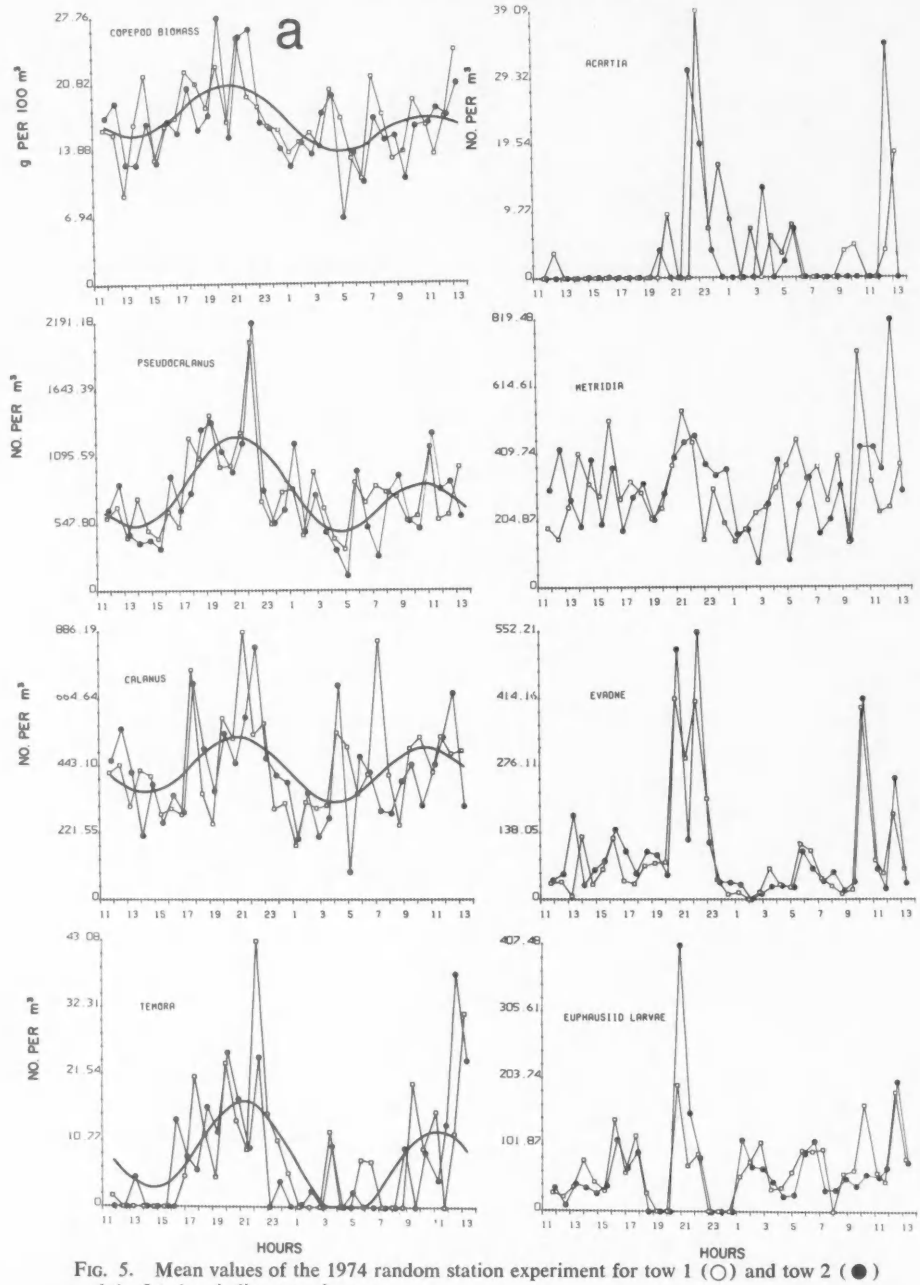

FIG. 5. Mean values of the 1974 random station experiment for tow 1 (○) and tow 2 (●) and the fitted periodic regression curve.

tically towed net was always 2 m above the bottom. Therefore, animals that were <2 m from the bottom would not be captured unless they vertically migrated. Several species showed a distinct increase in numbers during the night hours, which suggested they undergo vertical migration. In 1973 *Temora, Calanus, Sagitta,* and the shrimp *Pandulus* appeared to increase in the samples at night. In 1974 on Roseway Bank, only *Thysanoessa longicaudata* and *Tomopteris* showed a distinct increase in the night samples, while on the

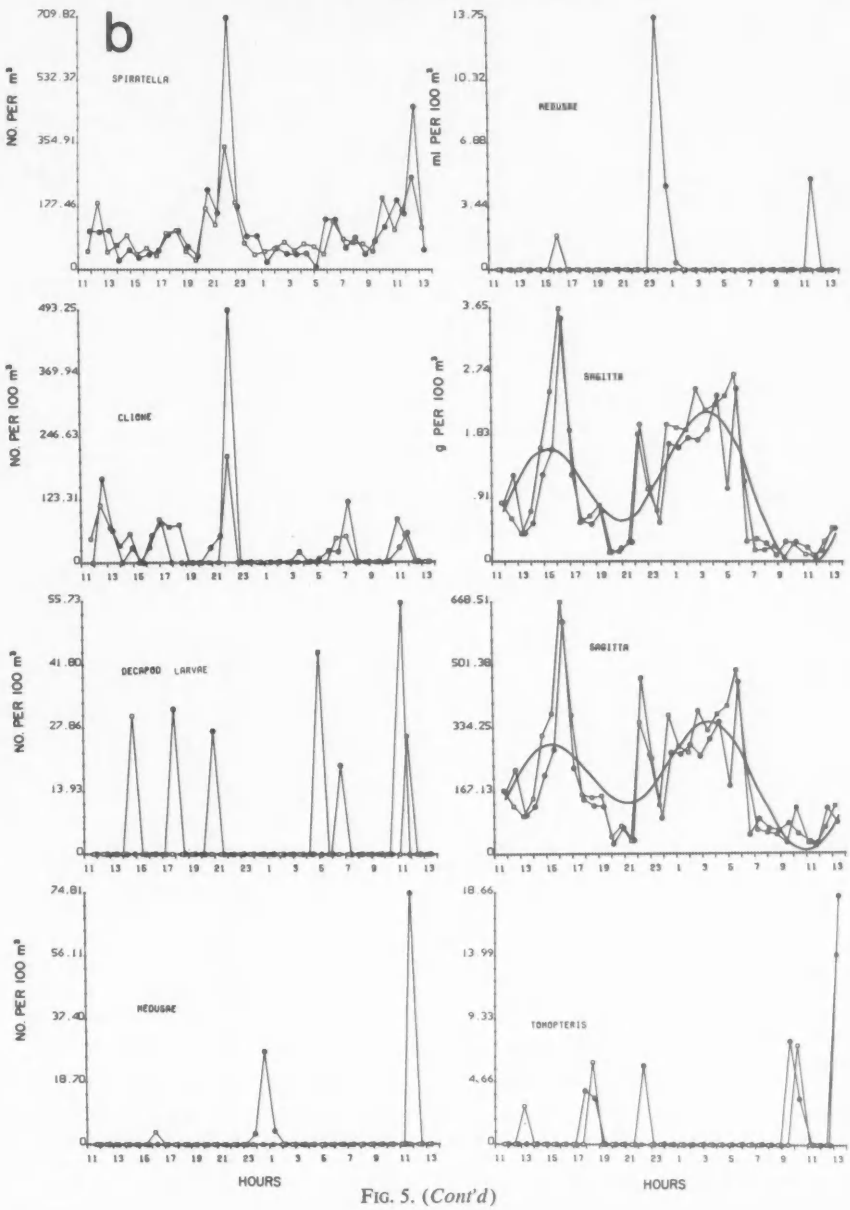

FIG. 5. (*Cont'd*)

random stations *Acartia* and *Thysanoessa longcaudata* increased at night.

RANDOM STATION EXPERIMENT

Means and variances between the samples from the random stations taken at the same time intervals as the earlier 26-h experiments were compared. In this experiment time and station were synonymous, since a new station was sampled at each new time.

All 21 sample components had a significant difference between the different hours, but only 4 had a significant difference between the tows (Table 1).

The copepod biomass, *Calanus, Pseudocalanus*, and *Temora* numbers had a periodic fluctuation (Fig. 5a) similar to those seen on Roseway Bank during the same cruise. *Sagitta* numbers and biomass (Fig. 5b) fluctuated with a greater amplitude than was seen on Rose-

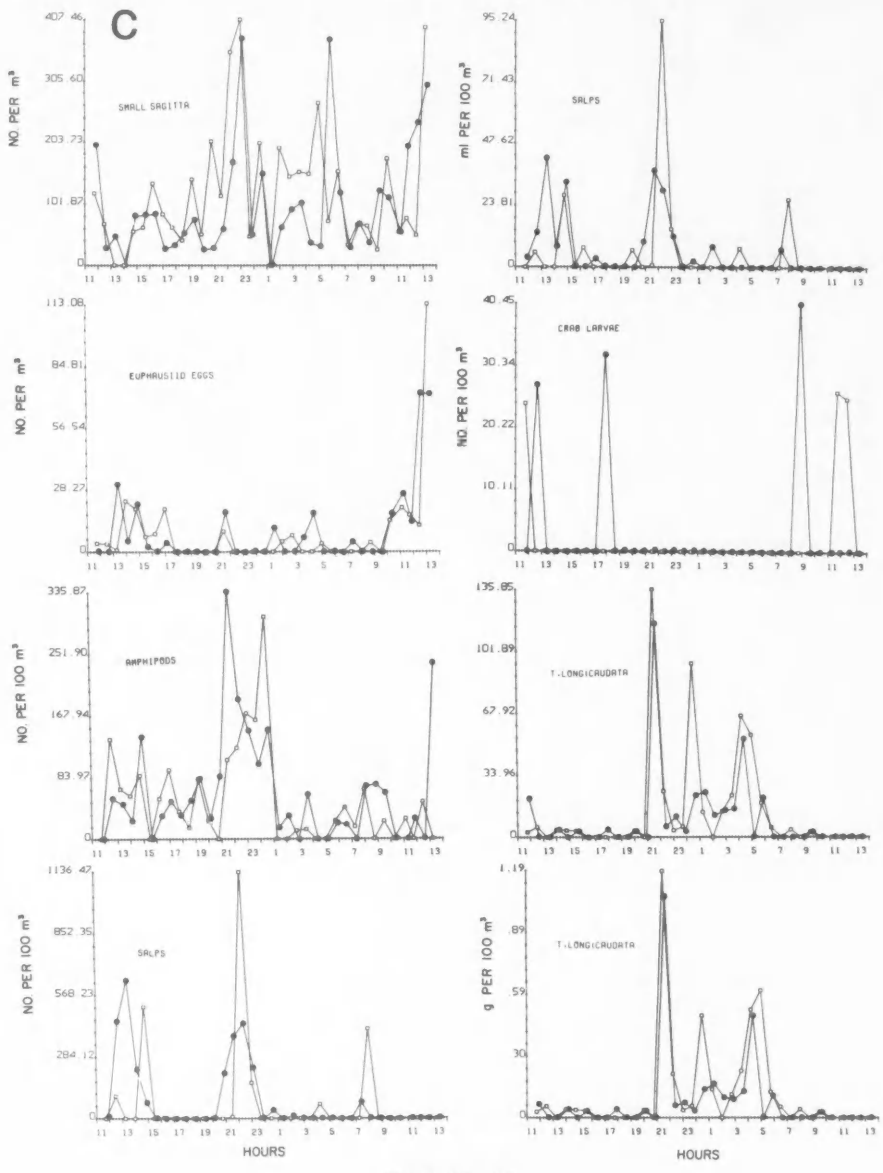

FIG. 5. (*Cont'd*)

way Bank. None of the other zooplankton components, other than silver hake eggs (Fig. 5d), showed a significant periodicity, but *Acartia, Evadne*, and *Spiratella* (Fig. 5a and b) did show large increases in numbers even though the analysis did not indicate periodicity.

All species showed fluctuations in numbers on different stations similar to those found at different times on the fixed stations. The similarity in the fluctuations indicated that the stations were located on animal densities, similar to those sampled over 26 h on fixed station B (Fig. 1).

SAMPLE VARIATION

The two experiments on Roseway Bank showed a greater reduction in their variance due to the periodic

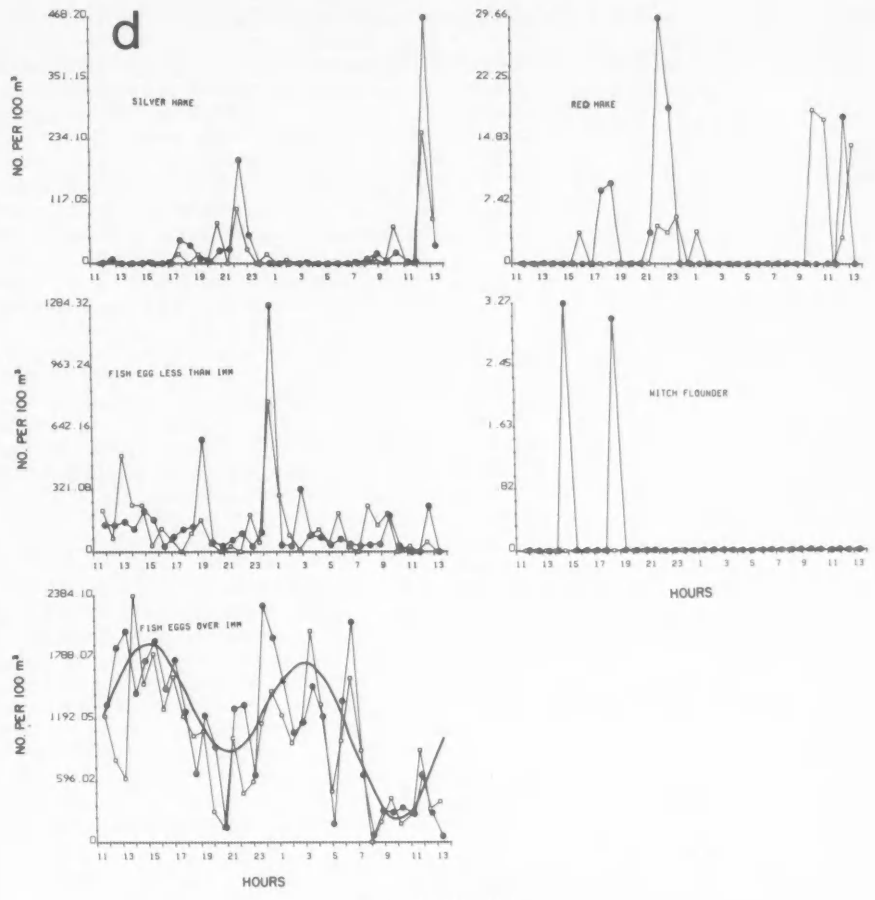

FIG. 5. (*Cont'd*)

regressions than the random stations experiment (Table 2). As much as 50% of the total variance was accounted for by the periodic regression in many of the species.

The majority of the variance for most components was random and unaccounted for by the regression equations. In most species there was no significant difference between tows, whereas there was between times. This means that small patches of zooplankton were moving past the station at a frequency greater than the sampling rate of 45 min, but at a slower rate than 4 min between tows.

The confidence limits for the Roseway Bank and random stations experiments (Table 3) was narrower than those for the shelf experiment. This was due to two factors: a smaller number of samples were taken during the shelf experiment, and a larger variation between samples as a result of the greater distance between shelf stations than between the random stations. The narrowest confidence limits in all experiments generally were found for the copepod and *Sagitta* biomass data.

The confidence limits for the different zooplankton components became wider as the sampling area became larger, with the shelf stations generally having components with widest limits.

The homogeneity of the variances of each of the zooplankton components in each 1974 experiment were compared with their counterparts in the other experiments. It was found that 42% of the components had a greater variance on the random stations than the Roseway station, that all zooplankton components of the shelf stations were significantly more variable than those on Roseway Bank, and that all but five were more variable than those of the random stations. There was a significant difference between the variances of all sample components between the 1973 and 1974 Roseway experiments except for copepod biomass.

TABLE 2. Percentage of the total variance removed by the periodic regression analysis using up to 3 harmonics.

Species	Roseway 1973	Roseway 1974	Random stations
Sagitta <5 mm			26
Sagitta >5 mm	49	29	46
Sagitta	37	27	56
Copepod (biomass)	41	42	26
Calanus	69	62	27
Pseudocalanus	38	47	24
Temora			24
Metridia			24
Oithona	18		
Euphausiid eggs		83	30
Euphausiid larvae		48	
Spiratella		23	
Evadne		21	
Thysanoessa longicaudata		52	
T. longicaudata (biomass)		58	
Fish eggs (red hake)			48
Amphipods			15

The means of each zooplankton component in each of the three 1974 experiments were compared with their counterparts in the other experiments by means of a series of *t*-tests using the method for unequal variances (Steele and Torrie 1960). Between the Roseway Bank and random station experiments all but two components (*Thysanoessa longicaudata* and *Calanus*) were significantly different, but the number of zooplankton components significantly different decreased in the above experiments when compared with the shelf stations. This was the result of unequal sample numbers and greater variance found on the shelf stations, which meant that the difference between the means had to be larger to be statistically significant. There was no consistent positive or negative difference between the various zooplankton components on the three stations. Even though statistically significant differences between the means were found for many of the zooplankton components they were surprisingly small considering the large differences in area covered by the three sets of samples. Because of the large number of degrees of freedom in the *t*-tests, relatively small differences in the means of the different zooplankton components were statistically different even though the differences would not have ecological significance.

CORRELATION BETWEEN ZOOPLANKTON COMPONENTS

Correlation coefficients were calculated between all the zooplankton components within each of the experiments with the aim of finding common species group-

TABLE 3. Geometric means and the 95% confidence limits (C.L.) expressed as a percentage of the means. Species with an asterisk have values expressed per cubic metre, the rest are per 100 m^{-3}. Values indexed 1 or 2 are means that were not significantly different from means from the shelf stations. Values indexed 3 are means not significantly different from means of the random stations.

	Roseway 1973			Roseway 1974			Random			Shelf		
	$\bar{X}$	C.L. as % of mean		$\bar{X}$	C.L. as % of mean		$\bar{X}$	C.L. as % of mean		$\bar{X}$	C.L. as % of mean	
Species												
Sagitta (no.)	287	5	721	656[1]	9.3	429	1361	12	394	527	.3	735
Sagitta (biomass)	1.4	60	157	1.5[1]	60	155	.87	36	224	1.4	20	308
Copepods (biomass)	19.6	38	218	12.8	50	179	17.3	55	168	11.7	20	308
Silver hake larvae							3.6	.6	720	1	9	427
M. norvegica							1	71	136	1.3	8	448
T. longicaudata	1	69	140	2.4[1,3]	1.5	657	3	10	693	1.4	14	364
*Calanus**	59	3	573	366[1,3]	41	206	398[2]	36	223	276	1.8	643
*Pseudocalanus**	1188	9	426	575[1]	38	215	704[2]	34	231	769	2.8	594
*Temora**	4	1	660	4.6[1]	3	578	3	2	646	6	.2	738
*Acartia**	1	43	198	1	59	158	3	5	527	2	2	619
*Oithona**	184	10	414	—	—	—	—	—	—	—	—	—
*Centropages**	183	9	433	12[1]	4	541	6[2]	1	654	9	.2	736
*Podon**	1	40	208	3[1]	19	321	4[2]	14	363	4	.01	590
*Evadne**	1	54	171	10	3	582	50[2]	2	363	43	.03	657
*Spiratella**				22	5	512	75[2]	12	393	45	.1	728
Euphausiid eggs*				8	.4	732	3	2	632	26	.09	717
Euphausiid larvae*				138[1]	15	142	27	5	723	79	.009	562
Fish eggs >1 mm (Silver hake)				1	36	224	676	2	619	2	1	669
Fish eggs <1 mm (red hake)				1	36	223	29	.05	692	2	2	611
Clione larvae				2.6	8	706	3	.02	737	1	31	244

TABLE 4. Correlation coefficients (r) between zooplankton components showing significant r values ($P < .001$) for the 1973 Roseway Bank experiment.

	Silver hake larvae	*T. longicaudata*	*Metridia*	*Sagitta* <5 mm	Copepod biomass	*Pseudocalanus*	*Sagitta* biomass	*Calanus*	*Evadne*
Sagitta <5 mm	.4437	.7095							
Pseudocalanus					.5689				
T. longicaudata				.7095					
Eurytemora									.4697
Calanus			.3925			.3975	.4694		
Sagitta >5 mm					.4329		.8125	.6001	

ings that occurred in more than one of the experiments. The probability level chosen to decide significance was $P < .001$. This extremely low level was selected because the large number of comparisons made the chance of spurious correlations high.

The 1973 Roseway Bank data had 288 component combinations of which only 11 had significant correlations (Table 4), most of which were between copepod species. There were more significant correlations between the species components in the 1974 Roseway Bank and random stations experiments than the earlier Roseway experiment (Table 5).

Comparisons were made between species components in the shelf experiment and data on the concentrations of particulate carbon and nitrogen, chlorophyll *a*, and phaeophytin measured at 5 m on each of the shelf stations at the same time as the zooplankton samples were collected. These data were taken from Subba Rao (1975). Out of a possible 220 correlations 37 were significant at $P < .001$. None of the species were significantly correlated with the four above factors, but all four factors were significantly correlated with each other (Table 6).

The significant correlations in each of the experiments were compared to find species correlations common to all. In the 1974 experiments the only species correlated in all were *Calanus*, *Pseudocalanus*, *Metridia*, and *Spiratella*. Out of these four species only *Calanus* and *Pseudocalanus* were significantly correlated in the 1973 experiment.

The auto- and cross-correlation analysis of the 1974 Roseway data found five zooplankton components significantly correlated with the tide when it was lagged 0–2 time units (45 min each). There was no correlation beyond 2 time unit lags. The 1973 data were not significantly correlated with any of the tide time lags. The wave length of the periodic regressions of the zooplankton components varied from 11.0 to 16.7 h (Table 7). Copepod biomass and *Pseudocalanus* were the only components in the 1973 data on which an estimate of the regression wavelength could be made. The wavelengths of both were about one half those found in 1974. Since *Pseudocalanus* was the dominant species it had a great influence on the wave form of the copepod biomass causing the two to have similar wavelengths. The wavelengths for the other components that had significant periodic regressions were greater than 26 h and therefore no reasonable estimate of the wavelength could be made because of the short time series.

DENSITY RATIOS OF ZOOPLANKTON

The ratios between the high and the low points on the calculated periodic regression curves were calculated for the more abundant species. Species that had curves with zero values were not included in these calculations because the ratio would have been infinity. The ratio between the highest and lowest mean values of two tows was also calculated to obtain an index of the difference in density between the high and background concentrations of animals. The curves provided an average ratio for the larger concentrations and the background, while the ratio from the actual mean value gave an indication of the extreme differences encountered between the small random concentrations within the larger concentrations.

The ratios (Table 8) indicated that the copepod biomass fluctuations on different stations were lower and more stable than the ratios for *Sagitta* biomass. *Sagitta* biomass ratios were higher on the random stations than the fixed stations. The ratios for the different copepod species were higher than those for the biomass, and except for the 1973 Roseway Bank data, were similar. The ratios calculated from Sameoto's (1975) data were slightly lower but within 50% of those of this study.

These results indicated that even though the relative numbers of animals changed in the different areas the ratio between the high concentrations and the background density was relatively stable for most species of copepods.

TABLE 5. Correlation coefficients (r) between zooplankton components showing significant r values at $P < .001$ for the 1974 Roseway Bank station and the random experiment.

Roseway Bank Random stations	Euphausiid eggs	Silver hake eggs	Red hake eggs	Silver hake larvae	Copepod biomass	*Pseudocalanus*	*Sagitta* biomass	*Calanus*	*T. longicaudata*	*Metridia*	*Spiratella*	*Sagitta* <5 mm	*Clione* larvae	*Tomopteris*	Tide
Euphausiid eggs								−.4475	−.5912		−.5031				
Euphausiid larvae	.4395					.4998		.6355		.5919					
Silver hake		−.4472													
Copepod (biomass)				.5044					−.5542						
Pseudocalanus					.6559					.7298	.6828				−.6175
Acartia		.5506						.6907							
Calanus				.6907	.7060	.6717				.5495	.7155				−.6787
Temora			.4404	.6554		.5660		.5172							
Sagitta (no.)		.5449					.9378								
Thysanoessa														.5604	
Metridia				.6531	.5654	.6388		.7978			.6403				−.4544
Spiratella				.7531	.4201	.6799		.8104		.7965					−.6059
Sagitta <5 mm				.4880						.5037	.4831				
Red hake				.6338	.4282	.5582		.5360		.6217	.6910	.5113			
Salpidae (mL)											.4907		.4338		

TABLE 6. Correlation coefficients (r) at $P < .001$ between zooplankton components and four inanimate components for the shelf stations.

	Sagitta	Decapod larvae	Red hake	Salps	*T. raschii*	*T. inermis*	Silver hake	*M. norvegica*	*Pseudocalanus*	*Acartia*	Euphausiid eggs	*Eurytemora*	*Sagitta* biomass	*Calanus*	*Sagitta* no.	*Metridia*	Particulate carbon	Particulate nitrogen	Chlorophyll *a*
Silver hake eggs			.6225																
Euphausiid eggs		.6750																	
Amphipods								.4103											
T. raschii								.6010											
Red hake eggs			.3985																
M. norvegica				.4703	.4626														
Eurytemora											.3920								
Medusae			.5297						−.5428		.7230	.5466	.5640						
Calanus									.5492										
Sagitta (no.)	.3943					.4565		.5854		−.4355			.6188						
Metridia									.6214					.5907					
Temora						.4121													
Shrimp						.4478									.5932				
Thysanoessa longicaudata				.4097	.6302		.6790	.6255											
Spiratella									.4567					.3974		.5713			
Particulate nitrogen																	.8218		
Chlorophyll *a*																	.5895	.7935	
Phaeophytin																	.4382	.4606	.4034

Discussion

The interpretation of periodic regression analyses on data series as short as those in this study should be viewed with caution. Ideally, the time series should include several cycles of the main frequency (Platt and Denman 1975) but unfortunately this was impractical in this study. The analyses indicated that periodic fluctuations of varying lengths did occur in numbers of most species over 26 h; these varied from a half to over a day in length. The similarity between many of the species fluctuations and the tidal periodicity suggested the tidal movement past the fixed stations was the main driving force that caused the periodic variation in animal numbers in 1974. The tidal move-

TABLE 7. Periodic regression wavelengths and correlation coefficients with the tide calculated from the autocorrelation analysis for the 1973 and 1974 Roseway stations. Degrees of freedom: 0 min = 33, 45 min = 31, 90 min = 30.

	Wavelength hours		Tide time lag 1974 (min)		
	1973	1974	0	45	90
Euphausiid larvae		16.7	−.35	−.40	−.41
Copepod (biomass)	7.5	16.0			
Pseudocalanus	8.0	13.0	−.52	−.56	
Calanus		15.0	−.63	−.65	
Temora		14.0	−.32	−.44	−.45
Metridia		11.0	−.40	−.44	−.45
Sagitta (biomass)		14.0			
Spiratella		14.0			

ment brought a range of zooplankton densities past the station and it is possible that some of the larger concentrations of species were brought back past the station by the elliptical movement of water on more than one tidal cycle. This would cause a similarity in animal number and tidal periodicity, which would probably occur only for a few tidal periods because of other ocean currents moving the plankton concentrations away from the station. Therefore, had a longer time series been taken, the fluctuations might have shown a changing frequency as different concentrations of plankton were moved past the station, but fluctuations would probably always occur as indicated by these experiments and those by Sameoto (1975).

The fluctuation pattern of the zooplankton numbers and biomass on the fixed station in 1974 was similar to that found by Sameoto (1975) in St. Margaret's Bay. He showed a close relationship with the fluctuation of zooplankton and the tide. In the 1973 station, there was no coherence between the tidal fluctuations and zooplankton components. But many did have wide fluctuations in their numbers, with the copepod biomass having a period fluctuation resembling that found in St. Margaret's Bay (Sameoto 1975). These results indicated the possible existence of a general pattern in the variation of zooplankton numbers on a fixed geographic station on the Scotian Shelf.

Few zooplankters were significantly correlated with each other on both fixed stations on Roseway Bank, while significant correlations between zooplankters were more common on the random stations and the shelf stations. This indicated that in a small geographic area, such as sampled on the fixed stations, the sample to sample variation tended to mask correlations between species. Whereas at stations taken over a wider area there was a greater likelihood of encountering species densities that varied by orders of magnitude, therefore making the probability of detecting significant correlations higher.

The larvae of silver and red hake had a high correlation with species of copepods on the random stations but not the fixed station. The lack of a significant correlation between the fish eggs and copepods suggested that when the young fish larvae hatch they may have to seek out concentrations of copepods. Unfortunately the nets used were not fine enough to retain copepod eggs and nauplii, a probable source of food for fish larvae (Marak 1960). Had this information been available the relationships may have been different.

The lack of positive correlation between any of the zooplankton components and chlorophyll *a*, phaeophytin, particulate carbon, or particulate nitrogen was surprising. It was expected that at least one of these would have reflected the food supply of some of the zooplankters and therefore be correlated with them. The four above factors were measured only at 5-m depth; since the zooplankton numbers represented the entire water column it is possible that these methods of sampling were not sensitive enough to detect relationships if they existed. Another reason for the lack of correlation may have been that zooplankton populations reflected the feeding conditions in the past.

It was possible to estimate the approximate range of patch sizes of the different species on the Roseway Bank stations by assuming a mean current speed on the slope of 7 km·d^{-1} (Forrester 1971; Sutcliffe et al. 1976) and measuring the time in which a major fluctuation passed the station. Depending on the species the times ranged from 6 to 15 h in 1973 and 9 to 12 h in 1974. This meant the concentrations in 1973 may have ranged between 1.7 and 4.4 km in width and in 1974, 2.6 to 3.5 km in width. The approximate size of the area of highest concentration of copepod species and small *Sagitta* on the random stations appeared to be between 2.5 and 4.0 km. These size estimates agree well but unfortunately totally ignore the influence of the tide and therefore can only be considered as tentative. Concentrations of zooplankton measured by Sameoto

TABLE 8. Ratios between the density of animals in patches and background density of animals. c, ratio between the peak of the curve and the low point of the calculated curve; r, ratio between the highest and the lowest mean of a pair of tows; A and B refer to two different stations (Sameoto 1975).

Zooplankton component	Roseway Bank 1973		Roseway Bank 1974		Ransom stations 1974		St. Margaret's Bay 1971 A		St. Margaret's Bay 1971 B	
	c	r	c	r	c	r	c	r	c	r
Copepod biomass	3.3	7.3	1.7	2.3	1.6	2.4	1.7	2.2	1.4	2.0
Sagitta biomass	6.4	14.6	2.3	5.1	43.5	44.9				
Mean and SE of copepod species ratios	7.3±2.87	10.2±3.98	3.0±.55	4.1±.66	4.4±.97	11.8±5.95	2.3±.16	6.7±1.91	2.2±.16	5.9±.56

(1975) in St. Margaret's Bay were at least 1.5 km wide.

The density ratios implied that there may be narrow limits to the ratios between the dense concentrations and the lighter background densities for copepods. All the data given were collected in August and September, and it is not known if similar ratios would be found during other months. Sameoto (1975) gave ratios for copepods collected in May which were very similar to those in this study. Wiebe (1970) found the density of zooplankton in patches varied between 2.6 and 5.1 times the background density, which are similar to the ratios found in this study.

The samples taken with the oblique tows on the shelf stations produced quantitatively and qualitatively similar samples to the vertical tows. The variances were larger on the shelf stations, but this was probably not the result of the method of towing, but rather due to the wide geographic area over which the stations were located. This view is supported by the fact that the random stations had a greater variance for most species than did the fixed station even though both sets of samples were taken by vertical tows.

The difference between most zooplankton components on Roseway Bank in 1974 and the shelf stations was not significant. More means were significantly different between the random station and the Roseway Bank data than between these two and the shelf data. Generally as water moves past a fixed station a reasonably complete array of zooplankton concentrations encountered over the entire shelf would likely be sampled. Thus it may be possible to obtain as good an estimate of the population mean for many zooplankton species from one station on the shelf, as from sampling a large number of stations over a wide area, provided that a time series of samples was taken over a period of at least two tidal cycles.

Many factors influence what is perceived as reality with regards to the density and distribution of zooplankton. It is dependent on the type of net used, the method and speed of towing the net, the frequency of tows, the degree of avoidance, and the vertical migration behavior of the different species. It was seen in this study and that of Sameoto (1975) that animals that are known to migrate to the bottom during the day are less likely to be caught even when the net is lowered to the bottom. As a result of these factors the estimates of the densities of some species are probably more accurate than others. Until more data are available about the degree of net avoidance and the migration behavior of different species, we will never be sure how close the estimates are to the actual numbers of animals present.

Acknowledgments

I would like to thank L. O. Jaroszynski and D. Rudderham of the Bedford Institute of Oceanography for their able assistance in the collection and analysis of the data and Dr R. J. Conover of the Bedford Institute of Oceanography for his helpful criticisms of the work.

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Revision of the Turbellarian Genus *Ectocotyla* (Seriata, Monocelididae) Associated with the Crabs *Chionoecetes opilio* and *Hyas araneus*

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FLEMING, L. C., AND M. D. B. BURT. 1978. Revision of the turbellarian genus *Ectocotyla* (Seriata, Monocelididae) associated with the crabs *Chionoecetes opilio* and *Hyas araneus*. J. Fish. Res. Board Can. 35: 1223–1233.

Ectocotyla hirudo (Levinsen, 1879) is redescribed and recorded for the first time from the gills and branchial chambers of *Chionoecetes opilio* and *Hyas araneus*. Problems arising from previous descriptions are resolved through comparison of freshly collected material, at different times of the year, with type material of *Coleophora* sp. and the type material of *E. paguri* Hyman, 1944, both of which are shown to be identical to the present material. Accordingly, *E. paguri* Hyman, 1944 is regarded as a junior synonym of *E. hirudo* (Levinsen, 1879). A new species, *E. multitesticulata*, is described and recorded also from *C. opilio* and *H. araneus*. *Ectocotyla multitesticulata* differs from *E. hirudo* primarily in the larger size of the worm, the greater number and size of the testes, the size of the egg capsule, and in the differences of the karyotype. The genus *Ectocotyla* is discussed in relation to closely related genera (*Peraclistus*, *Minona*, and *Preminona*) in the family Monocelididae.

Key words: turbellarian systematics, Proseriata, Monocelididae, *Ectocotyla*, crab commensal

FLEMING, L. C., AND M. D. B. BURT. 1978. Revision of the turbellarian genus *Ectocotyla* (Seriata, Monocelididae) associated with the crabs *Chionoecetes opilio* and *Hyas araneus*. J. Fish. Res. Board Can. 35: 1223–1233.

Les auteurs redécrivent *Ectocotyla hirudo* (Levinsen, 1879) et le signalent pour la première fois dans les branchies et les chambres branchiales de *Chionoecetes opilio* et de *Hyas araneus*. En comparant du matériel fraîchement recueilli à différentes époques de l'année avec du matériel type de *Coleophora* sp. et d'*E. paguri* Hyman, 1944, tous deux identiques au présent matériel, les auteurs résolvent les problèmes que posaient les descriptions antérieures. Ils considèrent donc *E. paguri* Hyman, 1944 comme synonyme junior de *E. hirudo* (Levinsen, 1879). Ils décrivent une nouvelle espèce, *E. multitesticulata*, qu'ils signalent également chez *C. opilio* et *H. araneus*. *Ectocotyla multitesticulata* diffère de *E. hirudo* principalement par la taille plus grande du ver, des testicules plus nombreux et plus gros, la taille de la capsule ovigère et le caryotype. Ils examinent le genre *Ectocotyla* par rapport à des genres étroitement apparentés (*Peraclistus*, *Minona* et *Preminona*) dans la famille des Monocelididae.

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Two species of *Ectocotyla* Hyman, 1944 (Seriata, Monocelididae) were found associated with the gills and the branchial chambers of the decapods *Chionoecetes opilio* (O. Fabricius) and *Hyas araneus* (Linnaeus). One of these is identified as *Ectocotyla hirudo* (Levinsen, 1879), which although it has been reported several times and under several names, still lacks a good description. The other species is here described for the first time as *E. multitesticulata* n.sp. Large numbers of both of these worms were collected on a more-or-less regular, monthly basis over 3 yr, providing individual worms at various stages of maturation and thus affording the opportunity for comprehensive descriptions based both on live and well-preserved material.

In view of the existing confusion, the genus *Ectocotyla* is reviewed and a full description provided of both *E. hirudo* and *E. multitesticulata*.

Materials and Methods

COLLECTION

Ectocotyla specimens were obtained from gills and surrounding carapace of *Chionoecetes opilio* and *Hyas araneus*.

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Imprimé au Canada (J5006)

Chionoecetes opilio and some *H. araneus* were caught off the east coast of Newfoundland; other *H. araneus* were collected off the coast of Scotland in St. Andrews Bay. Most worms examined were removed from live crabs immediately after they were collected; some crabs were kept alive for 2–3 mo in aerated tanks of salt water (Instant Ocean) at 0–4°C for examination of worms at a future time.

Histology

Whole mounts were stained with Gower's carmine or acetic acid alum carmine. Specimens were embedded in wax, sectioned at 10 μm, and stained with Masson's Hematoxylin–erythrosin–saffron (Gurr 1963) or Mallory's triple stain (Pantin 1969). Sections were examined with a Wild M20 microscope and drawings made with the aid of a Wild drawing tube. The eggs of *Ectocotyla* were stained and cleared with lactophenol and 1% toluidine blue, and mounted with Permount on a glass slide.

Scanning Electron Microscopy

Prior to examination, specimens were fixed for two hours with 5% gluteraldehyde in phosphate buffer and postfixed for 1 h in 1% osmium tetroxide in phosphate buffer. They were then dehydrated through ethanol, and processed through the critical point dryer with Freon 13. The specimens were mounted on S.E.M. stubs, placed in an Edwards vacuum evaporator and coated with 6 in. (15.24 cm) of 8 mil (0.203 mm) gold wire. The coated worms were examined with a Cambridge S4-10 S.E.M. and photographed both with a polaroid and a 35-mm camera.

Systematic Section

Family Monocelididae Hofsten, 1907
Genus *Ectocotyla* Hyman, 1944
Synonym *Coleophora* Steinböck, 1932

Genus: *Ectocotyla* Hyman, 1944

Monocelididae with plicate pharynx in anterior third of body. Ovaries behind pharynx base. Testes ventral between pharynx and penis. Male copulatory bulb with muscular seminal vesicle leading into tubular eversible penis. Accessory prostatic organ with spicule in midline behind penis. Ventral, external vagina slightly to the left of midline, between penis and spicule. Two oviducts extend from ovaries to meet just in front of vagina forming a common oviduct that joins the vagina with the egg-laying pore. Tegument with dorsal microvilli, ventral and ventrolateral cilia interspersed with microvilli and subtegumental nuclei. Posterior adhesive disk.
Type species: *Ectocotyla paguri* Hyman, 1944, by monotypy.
Synonym of: *E. hirudo* (Levinsen, 1879).

Remarks

Hyman (1944) erected the genus *Ectocotyla* in a new acoel family, the Ectocotylidae. This genus is here ascribed to the Monocelididae for reasons that will be given in the discussion to come.

Ectocotyla hirudo (Levinsen, 1879)

Monocelis hirudo Levinsen, 1879 : 193
Monotus hirudo, Graff 1882 : 424
Coleophora hirudo, Steinböck 1932 : 328
Ectocotyla hirudo, Hyman 1964 : 251
Ectocotyla paguri Hyman, 1944 : 2

Description

General features — Up to 2.0 mm long by 0.2–1.0 mm wide depending on state of maturity and degree of contraction (Fig. 1a, b). Fixed worms fusiform with plump middle region due presumably to withdrawn pharynx. Ventral adhesive disk at posterior end. Worms translucent milky color when live; opaque when fixed.

Tegument — Body surface ciliated though not uniformly. Ventral and ventrolateral surfaces densely ciliated; interspersed with microvilli (visible under electron microscope). Dorsal and dorsolateral surfaces more densely covered with microvilli but with occasional cilia (Fig. 1c). Cilia and microvilli arise from narrow tegument visible under light microscope. Tegument remains colorless when stained with Gower's or acetic acid alum carmine. Nuclei subtegumental with cytoplasmic connections to the tegument passing through muscle systems. Circular muscles immediately beneath tegument; below these is a layer of longitudinal muscle. Longitudinal muscle layer larger and more obvious than circular muscle bundles. Latter not present uniformly throughout worm. Loose parenchyma below longitudinal muscle layer contains various parts of male and female reproductive systems. In most posterior part of worm, eosinophilic caudal glands open on surface to adhesive disk.

Nervous and sensory system — Brain anterior, granular mass with statocyst embedded in anterior portion (Fig. 1a, d). Statocyst with circumferential nuclei has two contained statoliths and accessory granules. Two main nerves extend from brain to posterior of worm in lateral position. No eyes.

Alimentary system — Anterior, plicate pharynx twice as long as wide, with external and internal ciliation, projects posteriorly. Circular and longitudinal muscles of pharynx found beneath ciliated tegument, arranged as elsewhere in body. Pharynx leads directly to gut through mouth situated immediately behind brain. Gut saclike, slightly lobed, extends from mouth backwards almost to posterior of worm in region of egg-laying pore. Gastrodermal cells present during much of year. At full maturity, prior to egg release, gastrodermis breaks down and enlarged gut lumen receives fertilized eggs while yolk is added to them from vitellaria.

Male reproductive system — Male system develops first, can be seen degenerating as female system starts to develop. Up to 100 testes (Fig. 1a, b) lie ventrally between pharynx and penis. Mature testes 49–67 μm by 33–49 μm. Small ducts lead from testes into two lateral vasa deferentia which separately enter male copulatory apparatus. Latter composed of muscular seminal vesicle leading into tubular eversible penis (Fig. 2). Posterior to penis lies the accessory prostatic organ comprising a roughly spherical vesicle and associated chitinous spicule or stylet. Gland cells open into vesicle, which opens to outside by small pore behind penis. Spicule, often referred to as excitatory organ, may be lost during copulation.

Female reproductive system — Two ovaries situated slightly behind base of pharynx in ventrolateral position. Largest number of eggs found in any worm is 21. Eggs have granular nucleoplasm with characteristic eosinophilic nucleolus. Vitellaria extend from just in front of ovaries to opposite posterior end of intestine, in dorsolateral bands of elongate cells with eosinophilic nucleoli. Vitellaria become filled with lipoidal substance which is released directly into gut during period of mature egg production. Ventral, external vagina anterior to, and slightly left of spicule (Fig. 3a). Two oviducts join ovaries with vagina just behind penis (Fig. 2, 3b). These ducts initially store sperm carrying it to ovaries but later function as oviducts carrying fertilized eggs (Fig. 3c). From junction of two oviducts and vagina, a common oviduct leads posteriorly, opening to outside through the egg-laying pore posterior to accessory prostatic organ. Eggs from ovaries pass along oviducts into common oviduct which also opens posteriorly into gut. During egg laying, eggs released from gut via the common oviduct and egg-laying pore. Well-developed glands surround posterior part of common oviduct and egg-laying pore, presumably contributing to production of egg capsule and stalk. Stalked, encapsulated eggs 0.32–0.49 mm (Fig. 3d) when released found on gill filaments and branchial chamber of host.

Distribution

This species has been recorded from

West Greenland (Egedesminde); on surface hairs of *Pagurus pubescens* Krøyer, described as *Monocelis hirudo* by Levinsen (1879).

Greenland; no host cited, described as *Monotus hirudo* by Vanhoffen (1897).

West Greenland and the English Channel (Plymouth); on surface hairs of *P. pubescens*, described as *Monocelis hirudo* by Wesenberg-Lund (1925).

Greenland; on mouthparts of *Chionoecetes opilio* (O. Fabricius), described as *Monocelis hirudo* by Steinböck and Reisinger (1930).

South Greenland; on mouthparts of *C. opilio*, described as *Coleophora hirudo* by Steinböck (1932).

USA (Ellsworth, Maine); on the surface of *P. pubescens*, described as *Ectocotyla paguri* by Hyman (1944).

Falkland Islands; no host cited, described as *Coleophora* sp. by Westblad (1952).

Canada (east coast of Newfoundland and Bay of Chaleur off New Brunswick); on gills and branchial chamber of *Chionoecetes opilio*, described as *E. hirudo* in this study.

Scotland (St. Andrews Bay); on gills and branchial chamber of *Hyas araneus*, described as *E. hirudo* in this study.

Remarks

Material collected in the present study and that of Hyman described as *Ectocotyla paguri* are identical to material described by Levinsen (1879) as *M. hirudo*. The worm described by Westblad (1952) from Bock's collection as *Coleophora* sp. was reexamined and will be mentioned in the discussion of this paper. Westblad's material is also identical to *M. hirudo* and the material described by Hyman (1944) as *E. paguri*. Although the type slides of *E. hirudo* (Levinsen, 1879) were not available in the present study, we agree with the suggestion of Marcus (1954) that *E. paguri* (Hyman, 1944) and *Coelophora* sp. of Westblad (1952) resemble *E. hirudo*. Hyman (1964) also suggested that her material was similar to that described by Levinsen (1879) as *M. hirudo*. In our opinion these worms are identical and, by reason of priority, the species of Hyman becomes a junior synonym of *E. hirudo* (Levinsen, 1879).

Hyman's type material of *E. paguri* was collected by Dr E. G. Reinhard from *Pagurus pubescens* in August 1940. We have reexamined the type material and although it is poorly preserved, in the light of good material collected in the present study, we were able to determine more accurately the morphology of this worm than was possible previously. Hyman (1944) described the tubular eversible penis as the female copulatory organ, and the accessory prostatic organ with its chitinous stylet as the penis. The "round, deeply staining masses" found in the central body region were identified as embryos in this same paper, although in a later paper Hyman (1964) attempted to correct this error when she reported that the "embryos" previously identified as those of *Ectocotyla* were ingested embryos of the host crab. Our reexamination of her type material shows that what she identified as "embryos" are the testes of the worm. Comparison of the type slides with our material shows that the worms were fixed after egg laying as yolk had been released into the enlarged gut cavity. The loose, vacuolated nature of the gut at this time caused Hyman (1944) to include the worms in the Acoela, a mistake she later corrected (Hyman 1964) when this species was reassigned to the Alloeocoela and the family Monocelididae. The structures Hyman calls the testes are the vitellaria and the

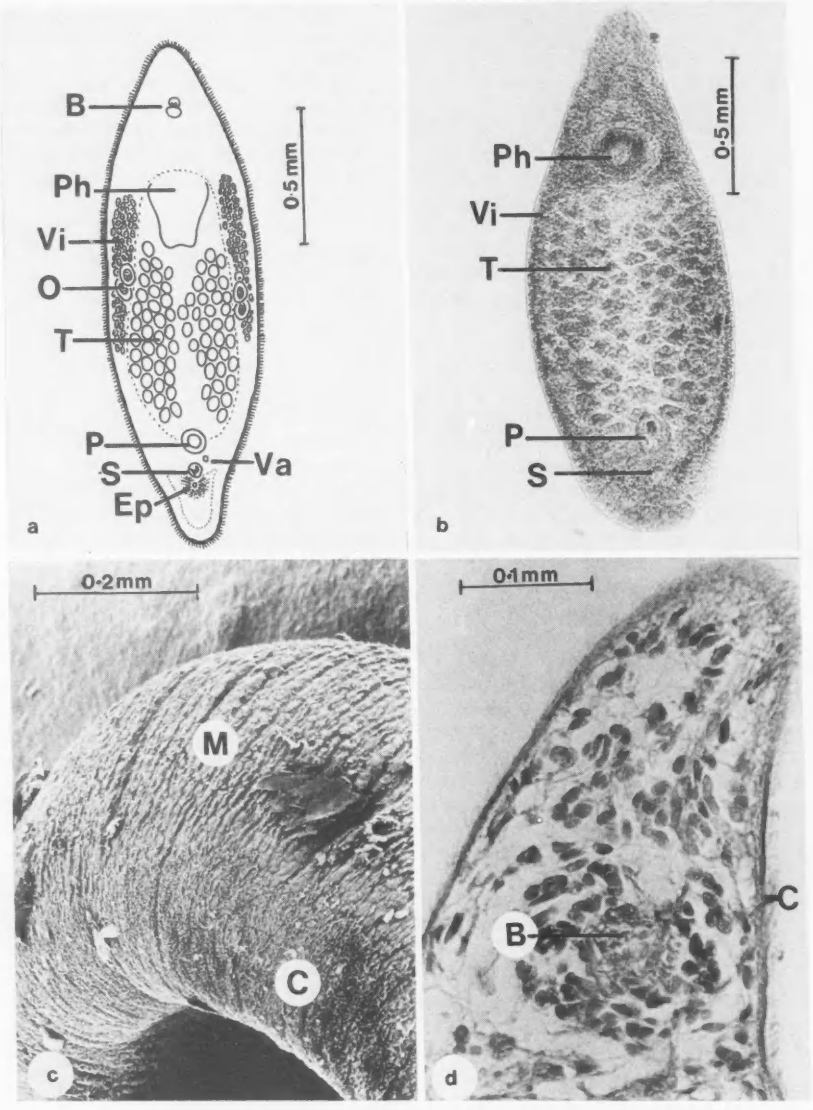

FIGURE 1. *Ectocotyla hirudo* (Levinsen, 1879).
a, Composite drawing of whole mount; b, photograph of whole adult worm; c, scanning electron micrograph showing a lateral separation between ventral cilia and dorsal microvilli; d, anterior end. (T.S.) B, Brain; C, Cilia; Ca, Capsule; COd, common oviduct; E, Egg; Ep, egg-laying pore; M, Microvilli; O, Ovary; Od, Oviduct; P, Penis; Ph, Pharynx; S, Spicule; St, Stalk; T, testes; Va, Vagina; Vi, Vitellaria.

real penis, stylet, and egg-laying pore are clearly visible in her type slides, although the egg-laying pore is mentioned by Hyman (1944) only as eosinophilous material. The vagina was not seen in the type material.

Hyman noted in her original description that although *E. paguri* was reported to be completely ciliated by Dr E. G. Reinhard, who observed the living specimens and from whom she received the preserved material, she found no traces of cilia in fixed specimens. Her type material does indeed appear as if much of

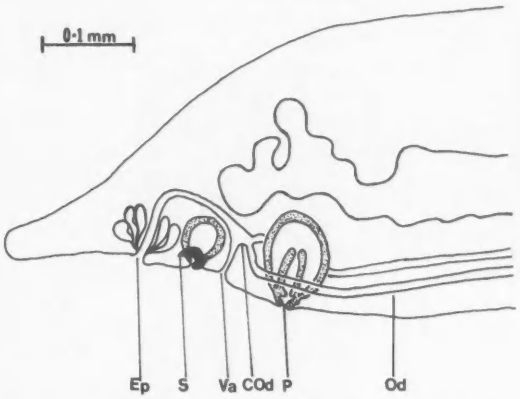

FIG. 2. Composite drawing to show relationship of male and female reproductive openings of *E. hirudo* (Levinsen, 1879). Abbreviations as in Fig. 1.

the tegument bearing cilia has sloughed off. She mentioned the "insunk" tegumental nuclei, found also in our material, and used this structure, typical of the Acoela, to support her erroneous consideration of this worm in that order. However, this feature is also characteristic of the Monocelididae which is included now in the Proseriata.

MATERIAL EXAMINED

R.M. (Riksmuseet, Stockholm): *Coleophora* sp. Westblad (1952). (1 slide of sagittal sections: The original slide and sections of Prof Sixten Bock).

A.M.N.H. 340; 341: *Ectocotyla paguri* Hyman, 1944. (2 slides: One of several whole mounts; one of a set of sagittal sections).

A.M.N.H. 920; 921: *Ectocotyla hirudo* (Levinsen, 1879); collected during the present study from *Chionoecetes opilio* (O. Fabricius) at about 73 m depth, off the coast of Newfoundland. (1 slide sagittal sections, dated February, 1975; 1 slide sagittal sections, dated June 28, 1975).

B.M.(N.H.) 1978.4.7.3-4: *Ectocotyla hirudo* (Levinsen, 1879); collected during the present study; all data as above.

Ectocotyla multitesticulata n.sp.

DESCRIPTION

General features — Up to 8.2 mm long by 0.16–1.64 mm wide depending upon degree of maturity and contraction (Fig. 4a, b). Fixed worms fusiform with plump middle region and with ventral adhesive disk at posterior end, used in attachment to host. Live worms translucent and milky color; fixed worms opaque.

Tegument — Body surface ciliated, though not uniformly. Ventral and ventrolateral surfaces densely ciliated; microvilli (visible only under electron microscope) interspersed between cilia. Dorsal and dorsolateral surfaces more densely covered with microvilli but with occasional cilia. Cilia and microvilli arise from narrow tegument visible under light microscope. Tegument remains colorless when stained with Gower's or acetic acid alum carmine. Nuclei subtegumental. Beneath tegument lies system of circular muscle. Larger layer of longitudinal muscle below circular muscles. Loose parenchyma below longitudinal muscle contains various parts of male and female reproductive systems. In posterior of worm, eosinophilic caudal glands open on to surface of adhesive disk.

Nervous and sensory system — Brain anterior, as granular mass with statocyst embedded in anterior portion. Statocyst, with circumferential nuclei, has two contained statoliths and accessory granules. Two main nerves extend laterally from [illegible] to posterior of worm. No eyes.

Alimentary system — Anterior plicate pharynx twice as long as wide, with external and internal ciliation; projects posteriorly. Circular and longitudinal muscles of pharynx beneath ciliated tegument, arranged as elsewhere in body. Pharynx leads directly into gut through mouth situated directly behind brain.

Gut saclike, slightly lobed, extends backwards almost to posterior of worm in region of egg-laying pore. Gastrodermal cells present for much of year. At full maturity, prior to egg release, gastrodermis breaks down and enlarged gut lumen holds eggs while vitellaria pass contents into gut.

Male reproductive system — Male system develops first, can be seen degenerating as female system starts to develop. Testes ventral, from pharyngeal region to male genital opening (Fig. 4a, b), greatest proportion lateroventral. Up to 590 testes occurring in three or four layers, 67–100 μm long diameter by 33–67 μm short diameter. Small ducts lead from testes into two lateral vasa deferentia that separately enter male copulatory apparatus. Male copulatory bulb comprises muscular seminal vesicle leading into tubular eversible penis. Posterior to penis a chitinous spicule, associated with roughly spherical vesicle, comprises the accessory prostatic organ. Gland cells open into prostatic organ, which opens to outside through small pore behind penis (Fig. 4c, 5).

Female reproductive system — Two ovaries situated lateroventrally and slightly behind base of pharynx. Up to 33 eggs in both ovaries.

Vitellaria in dorsolateral bands, extend from slightly in front of ovaries to posterior end of intestine. Common oviduct leads posteriorly from junction of vagina to gut with a branch to egg-laying pore. Vagina anterior to, and left of, spicule. Duct leads from vaginal opening to a point slightly behind penis. At this point, duct

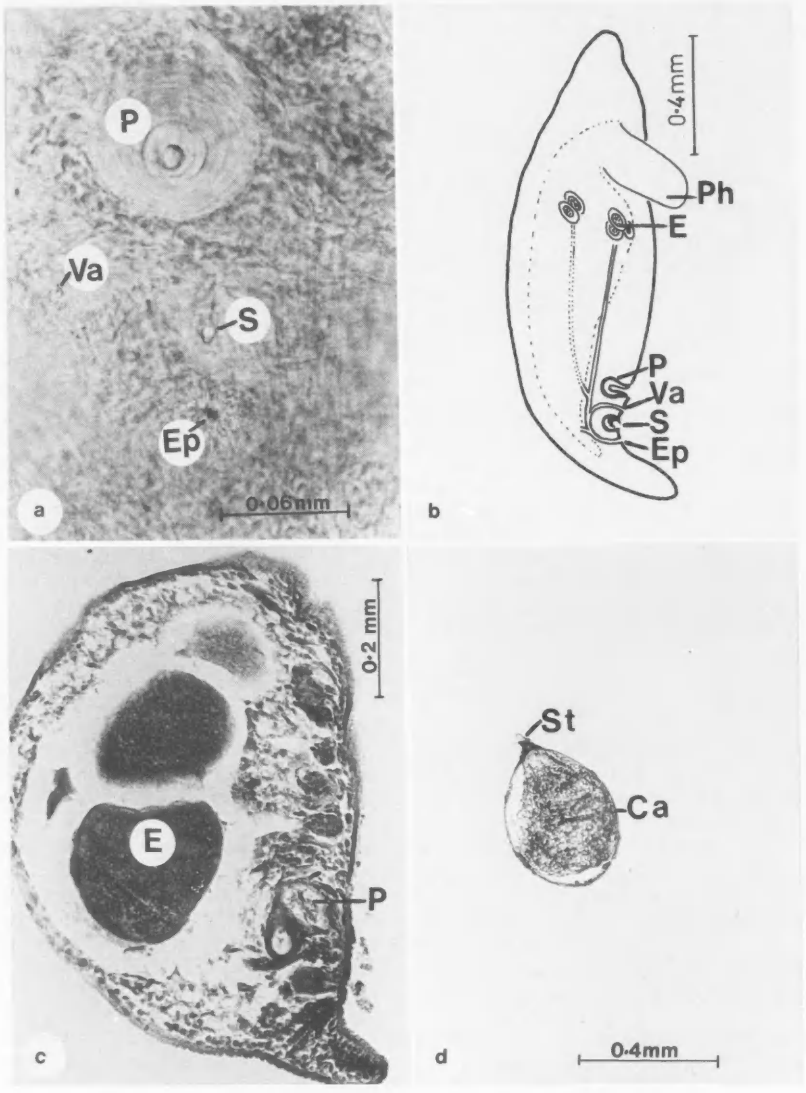

FIGURE 3. *Ectocotyla hirudo* (Levinsen, 1879).
a, Photograph of penis, spicule, vagina, and egg-laying pore; b, composite drawing of lateral view showing ducts and genital pores; c, photograph of adult (section) with eggs in gut prior to egg release; d, newly laid egg. Abbreviations as in Fig. 1.

joined by oviducts coming from each ovary. Egg-laying pore, posterior to stylet, surrounded by well-developed shell glands (Fig. 5). Duct leading to egg-laying pore open to gut at time of egg release. Gut therefore acts as uterus. No connection between gut and egg-laying pore exists during rest of life cycle. Stalked encapsulated eggs (Fig. 4d, e) 0.65–0.90 mm long diameter, found on gill filaments and carapace of branchial chamber. Most eggs about 0.73 mm long. Newly hatched worms (Fig. 4f) about 1.5 mm long.

DISTRIBUTION

This species has been recorded from

Canada (Atlantic Ocean, off east coast of Newfoundland; Bay of Chaleur, off New Brunswick); on gills

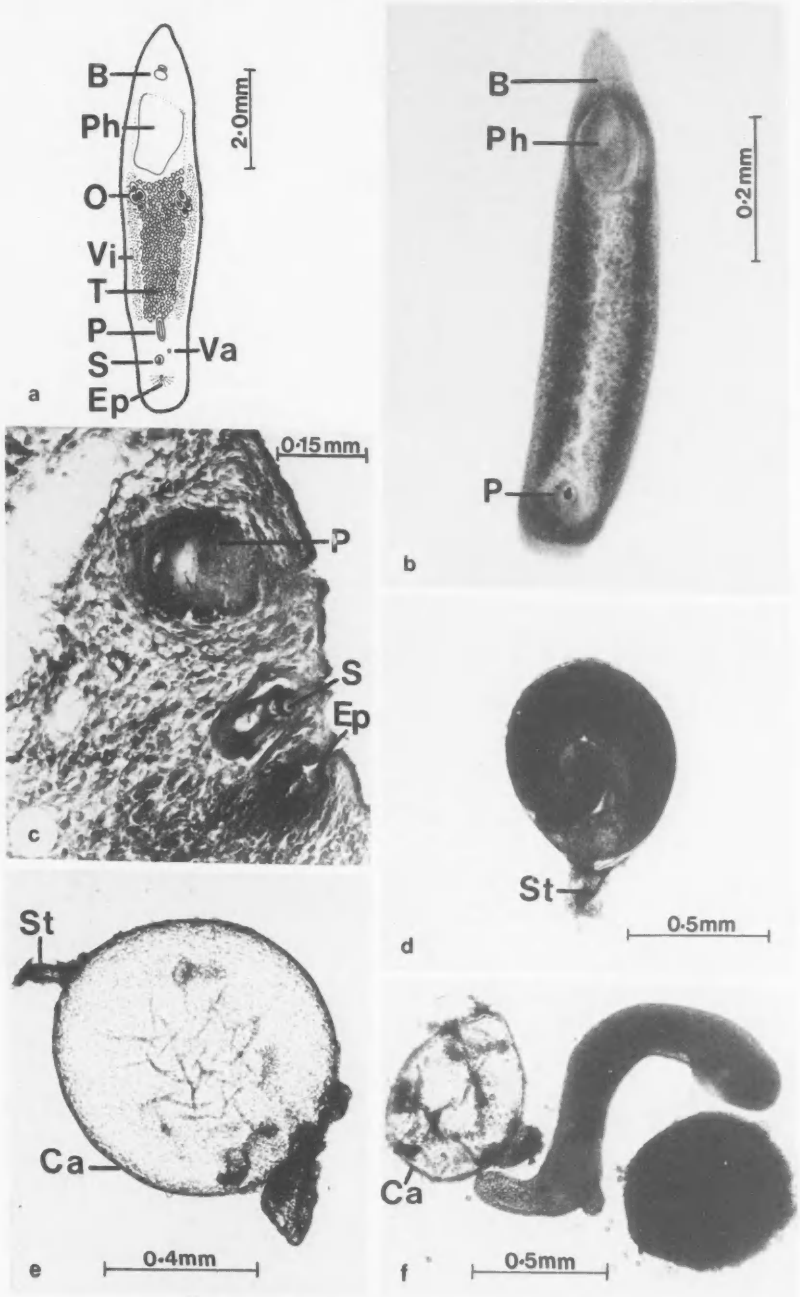

FIGURE 4. *Ectocotyla multitesticulata* n.sp.
a, Composite drawing of whole mount; b, photograph of adult, whole mount; c, sagittal, vertical section to show penis, stylet, and egg-laying pore; d, newly laid egg; e, fully developed egg with young worm; f, young worm just released from egg seen in Fig. 4e. Abbreviations as in Fig. 1.

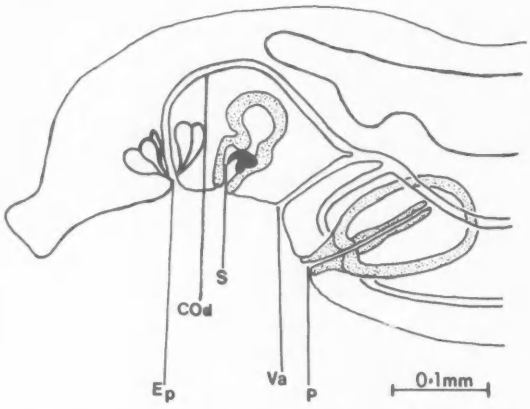

FIG. 5. Composite drawing to show relationship of male and female reproductive openings of *E. multitesticulata* n.sp. Abbreviations as in Fig. 1.

and branchial chamber of *C. opilio*; this study.

Canada (Bay of Chaleur); on gills and branchial chamber of *Hyas araneus*; this study.

REMARKS

This species closely resembles *E. hirudo* with which it is often associated on the gills and the carapace of the branchial chamber of the queen crab (*Chionoecetes opilio*), and *Hyas araneus*. The similarity between *E. multitesticulata* and *E. hirudo* is so close that initially the former species was regarded in this study simply as a population of older and, therefore, larger *E. hirudo*, the differences being considered as part of the normal size range. However, the differences seem sufficiently great and sufficiently constant to recognize the larger worms as a distinct and separate species.

TYPE MATERIAL OF *E. multitesticulata* n.sp.

Holotype: B.M.(N.H.) 1978.4.7.1; collected during the present study from *C. opilio* (O. Fabricius) at about 73 m depth off the coast of Newfoundland. Slide number: 124a, 124b. Size: 3.12 mm. Collected by L. C. Fleming and M. D. B. Burt, January 1973. Data on label: no. 124a or no. 124b/10 μm; *E. multitesticulata*; January 1973; *C. opilio*/Newfoundland; on gills of male; Masson's triple stain. Paratypes: B.M.(N.H.) 1978.4.7.2; collected as above. Slide number: 489. Size: 4.0 mm. Collected by L. C. Fleming and M. D. B. Burt, July 15, 1975. Data on label: no. 489/10 μm; *E. multitesticulata*; July 15, 1975; *C. opilio*/Newfoundland; from branchial chamber of male; Mallory's triple stain. A.M.N.H. 918; collected as above. Slide number: 200a; 200b. Size: 2.5 mm. Collected by L. C. Fleming and M. D. B. Burt, January 1974. Data on label: no. 200a or no. 200b/January 1974; *C. opilio*; male gill/Newfoundland; *E. multitesticulata*; Mallory's triple stain. A.M.N.H. 919; collected during the present study from *C. opilio* (O. Fabricius) from the Bay of Chaleur, off the coast of Caraquet, New Brunswick. Slide number: 275. Size: 2.75 mm. Collected by L. C. Fleming and M. D. B. Burt, May 26, 1974. Data on label: no. 275/10 μm; May 26, 1974; *C. opilio*/Caraquet, Bay of Chaleur, New Brunswick; *E. multitesticulata*; gill of female crab; Mallory's triple stain.

SPECIES RECOGNITION

Length of *E. multitesticulata* 4 times that of *E. hirudo*. Number of testes similarly 4 times greater. Testes of *E. multitesticulata* 67–100 μm long diameter by 33–67 μm short diameter; testes of *E. hirudo* 49–67 μm long diameter by 33–49 μm short diameter. Position of testes similar in both worms. One layer of testes in *E. hirudo*, four layers of testes in *E. multitesticulata*. Eggs of *E. hirudo* 0.32–0.49 mm long diameter; eggs of *E. multitesticulata* twice as long at 0.65–0.90 mm long diameter.

Eggs of both species found at all stages of development. Worms from larger eggs greater in size than those from smaller eggs. Differences in shape of eggs, as seen by comparing Fig. 3d with Fig. 4e, further serve to differentiate between the two species. Chromosome number the same in both species ($n = 6$). However, in *E. hirudo* there are five pairs of subtelocentric chromosomes and one pair of submetacentric chromosomes, whereas in *E. multitesticulata* all six pairs of chromosomes are submetacentric.

Discussion

HISTORY OF THE GENUS AND NOMENCLATURAL NOTES

A worm was first described from the surface hairs of *Pagurus pubescens* Krøyer by Levinsen (1879) who named it *Monocelis hirudo*. Although *Monocelis* was first erected by both Hemprich and Ehrenberg, it is now generally ascribed only to Ehrenberg (1831) who was editor of the series of works in which the genus was erected. *Monocelis hirudo*, again from *P. pubescens*, was redescribed by von Graff (1882) who transferred it to the genus *Monotus* (Diesing), which he revised to include part of *Monotus* Diesing, part of *Monops* Diesing, part of *Celidotis* Diesing, part of *Monocelis* Ehrenberg, and part of *Bdelloura* Leidy. Von Graff considered *Monotus* to contain those members of the family Monotida in which the female sex opening is located in front of the male opening. However, he wrongly assumed that the muscular bursa was the female organ and that the chitinous stylet was the male organ. Vanhoffen (1897) describing material from Greenland also recorded this worm as *Monotus hirudo* but did not indicate any host. Von Graff (1903) recorded this species, again from *P. pubescens*, as *Monocelis hirudo* and in 1913 he formally transferred the species back to the genus *Monocelis*, from *Monotus*, and provided redescriptions of the family Mono-

celididae (originally described by von Hofsten (1907)), the subfamily Monocelidinae (Midelberg 1908), and the genus *Monocelis*. In the revised family Monocelididae, von Graff included the family Monotida, the previous family Monocelididae (excluding *Plessisia* and *Hypotrichina*), and the family Automolidae (excluding *Otomesostoma*). *Monocelis hirudo*, from the hairs of *P. pubescens* in West Greenland, was also recorded by Wesenberg-Lund (1925) who also indicated their presence in the English Channel (Plymouth). *Monocelis hirudo* was reported from *Chionoecetes opilio* for the first time from South Greenland by Steinböck and Reisinger (1930) who found it on the mouthparts of the crab. They pointed out, however, that one of the characteristics of the genus *Monocelis* (ovary anterior to the pharynx) does not obtain in this case as the pharynx is anterior to the ovaries. In a later work, Steinböck (1932) transferred *Monocelis hirudo* and *M. alba* to a new genus *Coleophora* and described two new species of *Coleophora*. These new species, *C. chionoecetis*, from the eggs of *Chionoecetes opilio*, and *C. levinseni* from West Greenland, and the genus *Coleophora* itself are attributed by Steinböck (1932) to Fuegenschuh who was supposed to publish in Meddelser fra Dansk Naturhistorisk Kopenhagen but whose paper never appeared. Because of this, these species and the genus *Coleophora* have since been attributed to Steinböck himself.

Hyman (1944) erected the genus *Ectocotyla* which she placed in a new family, the Ectocotylidae. In this family she included *E. paguri* Hyman, 1944 which was found on the surface of the hermit crab *P. pubescens* and which she thought to be the first ectocommensal acoel turbellarian known. However, Westblad (1948) questioned the inclusion of *E. paguri* in the Acoela and pointed out that this species closely resembled *M. oofaga* Friedman, 1924 which had been recorded previously as an ectocommensal of *Hyas araneus*. In a systematic reorganization of the family Monocelididae, Marcus (1949) amalgamated *Togarma*, a new genus, *Peraclistus*, *Meidiama*, *Monocelis*, *Minona*, *Coleophora*, *Mesoda*, and *Archiloa* in the subfamily Monocelidinae, retaining the genus *Coleophora* for the species *C. hirudo*. The following year, Marcus (1950) questioned the inclusion by Steinböck (1932) of both *Monocelis hirudo* and *M. alba* in *Coleophora* as *M. hirudo* has an anterior pharynx while *M. alba* has a posterior pharynx. Marcus (1952) indicated that the genus *Coleophora* Steinböck should be replaced by *Ectocotyla* Hyman and in later paper (in the discussion following the description of a new species, *M. anta*) Marcus (1954) pointed out the resemblance between *M. hirudo* Levinsen and *E. paguri* Hyman as well as the similarity of their female organs with those of a *Coleophora* species described by Westblad (1952) from the Falkland Islands. Hyman (1964) in some corrective remarks on alloeocoels included *E. paguri* in the family Monocelididae and (like Marcus 1954) further pointed out its resemblance to *M. hirudo* in the anterior position of the pharynx, presence of a caudal disk, and its ectoparasitic habits. Hyman also indicated that although *M. hirudo* had been designated as the type of the genus *Coleophora* this name is preoccupied and should be replaced by *Ectocotyla* thus following Marcus (1952). More recently, Karling (1966) discussed the Monocelididae Hofsten, reorganizing the genera of this family according to the construction of the copulatory bulb and the presence or absence of an accessory prostatic organ. He further indicated the closer relationship between *Ectocotyla*, *Peraclistus*, *Minona*, *Preminona*, and *Duplominona*.

Ectocotyla hirudo has been reported on the mouthparts of *Chionoecetes opilio* (Steinböck and Reisinger 1930) but our study reports this species on the gills and in the branchial chamber of this crab for the first time. This is also the first report of *E. hirudo* on *Hyas araneus*. Examination of 20 specimens of *P. pubescens* did not reveal the presence of any turbellarians, although it should be noted that the hermit crabs did not come from the same locality as the *C. opilio* or the *H. araneus*.

Biological Notes on Reproduction

Gut — Although there is a superficial resemblance of the gastrodermis in the present material to that described by Willier et al. (1925) for *Planaria* (=*Dugesia*) *dorotocephala*, the relatively distinct boundary separating the gastrodermal cells from the parenchyma in their material was not evident at any time of the year in *Ectocotyla*. As indicated in the descriptions, the gut of *Ectocotyla* serves two disparate functions: (1) in digestion, as would be expected during the trophic stage when the worm is actively feeding; (2) after reaching full maturity and just prior to the release of eggs, the gastrodermis breaks down and the greatly enlarged gut lumen serves to retain fertilized eggs while yolk is being added to them.

Male reproductive system — As is evident from the historical review of *E. hirudo*, there has been considerable confusion in the past regarding which of the organs was the penis and which was the vagina. In his original description of *Monocoelis hirudo*, Levinsen (1879) described the penis as a hook-shaped chitinous stylet anterior to which is found a seminal bursa. Between these two organs there is a single opening with a duct to each. Von Graff (1882) confirmed this description (as *Monotus hirudo*) but added that in his estimation there must be a separate female opening situated more anteriorly. Although he repeated this description in 1913, again mentioning the curved chitinous stylet as a penis, we agree with Karling (1966) that this stylet is a part of an accessory organ. As indicated by Westblad (1952) and Karling (1966), the penis is the organ previously described as the seminal bursa, and is a tubular eversible structure sur-

rounded by a muscular seminal vesicle. The glandular vesicle associated with the stylet has been described as a prostatic organ (Karling 1966) or as an excitatory organ (Westblad 1952). The present study supports these views.

We know from the present study that *E. hirudo* and *E. multitesticulata* are protandrous hermaphrodites and that the testes mature well in advance of ovarian or vitelline development. Accordingly, not all parts of the female and the male reproductive apparatus may be present at the same time and, furthermore, the stylet which develops early is often absent in older worms presumably being lost during copulation.

Female reproductive system — Examination of the material described by Westblad (1952) as *Coleophora* sp., in combination with the present material of *E. hirudo*, indicates that they are both the same species. As Westblad's material is in a poor state of preservation, it is not surprising that a full and accurate description was not published. Careful examination of his material, however, indicates that the vagina does open, as in *E. hirudo*, to the left of the stylet (not elsewhere in the midline) and that the vaginal duct is joined immediately behind the penis, by the two oviducts coming from each ovary. These ducts, misnamed by Westblad as the "germovitelloducts," do not unite at a bursa seminalis as he suggested; what he presumably mistook as the bursa is the point where the common oviduct joins with the vagina as described in our material and from which the right and left oviducts lead to the ovaries.

As indicated above, the yolk empties into the gut, and does not enter the "ovovitelloducts" as some writers have suggested is characteristic of the Monocelididae (Marcus 1946, 1951; Beauchamp 1961), and of *Ectocotyla* in particular (Westblad 1952).

In *Ectocotyla* the sperm is stored in the oviducts after copulation, often near the ovaries, until the eggs are developed. It is more usual in the family Monocelididae for the "alien" sperm to be stored in a bursa near the vaginal opening (Karling 1966) or in an expanded portion of the common oviduct (Marcus 1949). However, *Promonotus villaceae* Marcus shows "precocious" insemination of an ovocyte in the ovary (Marcus 1949) thus indicating that sperm in this species, like *Ectocotyla*, move up the oviduct close to the ovaries. In addition, *Necia sopha* Marcus has no bursa and "alien" sperm were seen in the prepharyngeal part of the ovovitelloducts (Marcus 1950).

Minona divae and other monocelids such as *Monocelis fusca* possess elongated cells in the common ovovitelloduct which project through the intestinal epithelium into the gut lumen. This is the "resorbiens" type of bursa (Karling 1966) in which the bursa is an annex to part of the common oviduct and from which "alien" sperm can be transferred to the intestine.

Although the vitellaria are not released into the oviduct in *Ectocotyla*, the fertilized eggs pass down the oviduct, through the common oviduct, and eventually into the gut where they become surrounded by the yolky material of the vitellaria. The eggs are released through the shell glands and egg-laying pore. This use of the gut has not been found in any other of the Monocelididae to date, but the present study indicates that it is also true in *Peraclistus oofagus* and possible in other monocelids.

Karling (1966) states that an open communication between the oviduct and the intestinal lumen, a "genitointestinal pore" can be regarded as a kind of bursal organ. However, this duct in *Ectocotyla* does not qualify as a genitointestinal duct in the sense used by most writers.

The presence of a genitointestinal connection is regarded by some (Steinböck 1924; Reisinger 1930) as proof that the eggs of Turbellaria were originally discharged orally, the connection constituting the primitive oviduct. Bock (1927) and Meixner (1928) suggest that the connection is too variable to be considered as indicative that the original female canal ran from the ovaries to the intestine. Although Hyman (1951) agrees that turbellarian eggs were originally discharged orally, she does not accept the "sporadic occurrence of a genitointestinal connection" as proof of this being a primitive oviduct and suggests further that conditions in the Acoela argue against this idea.

Position of *Ectocotyla* Within the Monocelididae

Karling (1966) reorganized the genera of the Monocelididae according to the construction of the copulatory bulb and the presence or absence of an accessory prostatic organ. *Ectocotyla* Hyman, 1944; *Peraclistus* Steinböck, 1932; *Minona* Marcus, 1946; *Preminona* Karling, 1966; and *Duplominona* Karling, 1966 were considered to be closely related taxa within the Monocelididae as they all have an accessory prostatic organ with spicule.

Ectocotyla can be separated from the other above mentioned taxa as it has an anterior pharynx. If the positions of the stylet or spicule and the vagina are taken as the criteria of separation of genera, the following genera would be considered as distinct: *Minona*, which has a vagina opening anteriorly to the penis, with a postpenial spicule and a posterior egg-laying pore; *Peraclistus*, which has a postpenial vagina, located in the midventral line, a postvaginal stylet and a posterior egg-laying pore (these genera and the genus *Duplominona* are discussed at length elsewhere (Fleming and Burt 1978)); *Ectocotyla*, which also has a postpenial vagina but slightly to the left of the midline, a postvaginal spicule and a posterior egg-laying pore; *Preminona*, as described by Karling (1966), which has the accessory prostatic organ anterior to the penis and the vagina anterior to the prostatic organ.

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Ultrastructural Aspects of the Causative Agent and Renal Histopathology of Bacterial Kidney Disease in Brook Trout (*Salvelinus fontinalis*)[1]

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YOUNG, C. L., AND G. B. CHAPMAN. 1978. Ultrastructural aspects of the causative agent and renal histopathology of bacterial kidney disease in brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 35: 1234–1248.

The renal histopathology of bacterial kidney disease in both naturally and experimentally infected brook trout (*Salvelinus fontinalis*) was investigated here on the ultrastructural level. Abnormal changes in the glomerulus and renal tubule during the experimental infection are described chronologically and compared with the changes observed in the fully developed natural infection. These abnormalities are similar to those occurring in mammalian renal disease. Evidence of a host immune and inflammatory response is presented. The bacterial strains involved in the two infections are compared in cell size, internal morphology, and manner of division to help characterize the as yet unclassified causative agent.

Key words: morphology, fine structure, pathology, *Corynebacterium*, renal, bacterium, trout, disease, Salmonidae

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Nous avons étudié au niveau ultrastructural l'histopathologie rénale de la maladie bactérienne du rein chez des ombles de fontaine, *Salvelinus fontinalis*, infectés à la fois naturellement et expérimentalement. Nous décrivons, dans leur ordre chronologique, les changements anormaux qui se produisent dans le glomérule et le tubule rénal lors de l'infection expérimentale, et nous comparons ces changements avec ceux observés dans une infection naturelle pleinement développée. Ces anomalies sont semblables à celles que l'on observe dans la maladie rénale des mammifères. La réponse de l'hôte est l'immunité et l'inflammation, et nous en décrivons les signes. Afin d'aider à caractériser l'agent pathogène encore non classifié, nous comparons les lignées bactériennes responsables des deux infections, sous le rapport de la grosseur des cellules, de leur morphologie interne et de leur mode de division.

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THE histopathology of bacterial kidney disease (BKD) has been described, on the light miscroscope level, by various researchers, beginning with Belding and Merrill (1935), who noted that 100% of the brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) examined carried microscopic lesions of the kidney, and that to a lesser extent the liver and spleen were also affected. Most of the subsequent descriptions of BKD in salmon and trout have noted pathological changes in the kidney (Earp et al. 1953; Snieszko and Griffin 1955; Smith 1964), but some have not (Wood and Wallis 1955). Apparently the BKD process varies in several respects with relation to such factors as the species of fish, fish age, diet, and seasonal climate (Wood and Wallis 1955; Smith 1964; Snieszko 1972). Previous descriptions of the disease may have been complicated by the fact that other diseases involving the kidney, such as visceral granuloma and infectious pancreatic necrosis, may be present simultaneously with BKD in fish (Bullock et al. 1971; Yamamoto 1975). The above findings suggest that the histopathology of BKD is a complex process that may vary with environmental conditions and may involve, in spite of the name, various target organs. To understand better the morphological events of the disease process as it affects the

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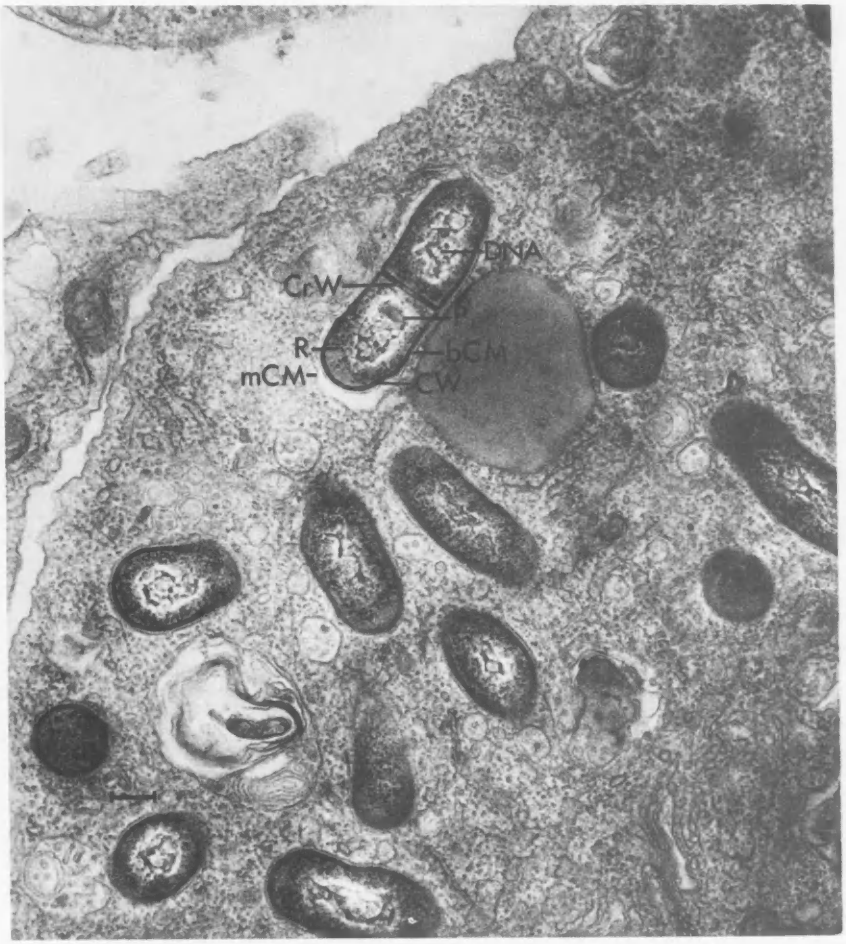

FIG. 1. Natural infection. Kidney disease bacteria located in macrophage cell of Lamar trout kidney. Macrophage cell membrane, mCM; deoxyribonucleic acid, DNA; peripheral bodies (mesosomes), P; ribosomes, R; bacterial cell wall, CW; bacterial cell membrane, bCM; cross wall of bacteria, CrW. Bar scale = 0.2 μm.

kidney, this study investigates, on the electron microscope level, the renal histopathology of BKD on a chronological basis in experimentally infected brook trout and compares these findings with those from a fully developed, naturally occurring infection.

The kidney disease (KD) bacterium has not yet been classified in a genus and species, although it has been suggested that it may be a corynebacterium (Ordal and Earp 1956; Vladik et al. 1974). Previous ultrastructural examination has shown only that the cell is a small bacillus found intracellularly in host tissues (Yamamoto 1975) and that it does not bear pili or flagella (Ordal and Earp 1956). While the phylogenetic classification of the bacterium may ultimately depend on biochemical methods, such as comparative DNA and protein sequencing (Schwartz and Dayhoff 1978) and on the biochemical analysis of cell wall composition (Barksdale 1970), a study of the cell's fine structure and division process will help in the characterization of the organism. This paper examines two strains of KD bacteria, the first, called the Lamar strain, as it appears within the host tissues of brook trout infected in nature, and the second, designated the 12.11 strain, after being harvested from culture media and as it appears in the fish tissues in an experimental infection.

Materials and Methods

Naturally infected brook trout, designated lot number

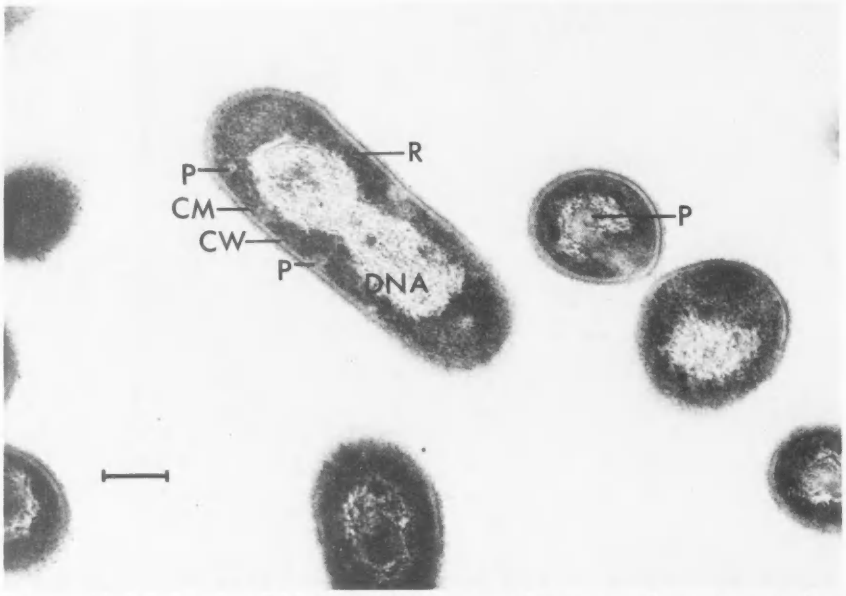

FIG. 2. Bacterial culture. Kidney disease bacteria of strain 12.11. The cell seen in longitudinal section is beginning to divide; other cells are seen in cross section. The cells show cell wall, CW; cell membrane, CM; deoxyribonucleic acid, DNA; peripheral bodies (mesosomes), P; ribosomes, R. Bar scale = 0.2 μm.

2UWVa, and aged approximately 17 mo, were obtained from the Federal Fish Hatchery at Lamar, Pennsylvania. BKD was diagnosed in these fish, hereinafter called Lamar fish, on the basis of gross symptoms and the presence in their tissues of many gram-positive bacteria resembling the classic description of the kidney disease pathogen. The diagnosis was later confirmed serologically by the methods of Chen et al. (1974) at the Eastern Fish Disease Laboratory (EFDL) of the U.S. Fish and Wildlife Service.

Five naturally infected Lamar brook trout exhibiting external disease symptoms were killed by decapitation. Thin, transverse sections of the kidney, spleen, and liver were collected for examination under the light microscope. These tissues were fixed in picric acid–Bouin's solution, embedded in paraplast, and sectioned at 6 μm on an American Optical Company Spencer 820 microtome with a steel knife. Sections were stained in Harris' hematoxylin and eosin, and also gram stained according to Taylor's (1966) method, and studied with a Zeiss binocular microscope. Additional specimens from the middle region of the kidney were prepared for examination under the electron microscope. These tissues were double-fixed in glutaraldehyde and osmium tetroxide according to the method of Millonig (1961), embedded in epoxy resin after the method of Luft (1961), cured at 60°C for 24 h, and sectioned on a Porter-Blum MT2-B ultramicrotome (Sorvall). Ultrathin sections were floated onto copper grids and double-stained with uranyl acetate and lead citrate, after the methods of Watson (1958) and Reynolds (1963), respectively. The sections were studied with an RCA EMU2-D electron microscope.

Brook trout to be experimentally infected with BKD were obtained from lot number 2YWaWS at the National Fish Hatchery in White Sulphur Springs, West Virginia. Fish at this hatchery are monitored for the presence of serum antibodies to BKD and were free of the pathogen up to the date of collection. Approximately 50 fish, aged about 15 mo, were divided into an experimental and a control group, and maintained in separate tanks of about 190 L (Westminster Scientific) at 14°C. The experimental fish were anesthetized with 0.01% MS-222® (Ayerst Laboratories) and injected intraperitoneally with 0.1 mL of a BKD strain (12.11) cell suspension with a concentration of 10^{10} cells/mL of phosphate-buffered sterile saline. Bacterial cells in the remaining inoculum were prepared for examination in the electron microscope by fixation in osmium tetroxide according to the method of Kellenberger et al. (1958). The specimen was then processed and examined in the electron microscope in the same manner as the trout tissues noted above. Bacterial strain 12.11 was obtained from the EFDL and had originally been isolated from spring chinook salmon (*Oncorhynchus tschawytscha*) at the Rapid River Hatchery in Riggins, Idaho. The strain was cultured by the EFDL at 15°C on Mueller-Hinton medium plus 0.1% L-cysteine. Shortly before inoculation, the identity of the bacteria in the inoculum was checked serologically by a slide agglutination test according to the method of Bullock et al. (1974). The control fish were anesthetized as described above and injected with 0.1 mL sterile physiological saline.

One control fish and one experimental fish were killed immediately prior to the inoculation and at intervals of about 3 d thereafter for 47 d (see Table 1). Tissue speci-

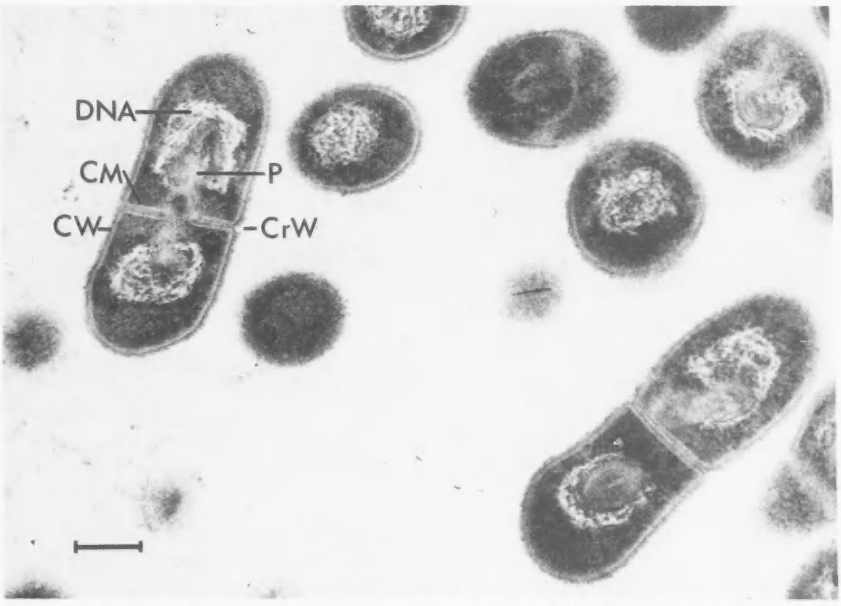

FIG. 3. Bacterial culture. Kidney disease bacteria of strain 12.11. One cell (at right of field) has recently completed the membrane septum and cross wall phases of cellular division, but the daughter cells have yet to separate. Another cell (at left of field) has nearly completed division, and the construction of its cross wall, CrW, is nearly completed. During cell division, a transverse cell membrane, CM, and cell wall, CrW, are laid down simultaneously. Deoxyribonucleic acid (DNA) and peripheral bodies (mesosomes) (P) are visible. Bar scale = 0.2 μm.

mens were collected and processed as described above, except that the paraplast-embedded tissues were fixed in Zenker's fixative.

Results and Discussion

THE BACTERIA

Consistent with previous reports (Bullock et al. 1971), the cell size in both strains examined here is approximately 0.3–0.5 μm in width by 0.6–1.0 μm in length. Mature Lamar strain cells (natural infection) in the process of cell division measure approximately 0.3–1.0 μm, and the daughter cells measure approximately 0.3 by 0.6 μm (Fig. 1). These cells are located in the cytoplasm of what appears to be a host macrophage cell, and each bacterium is surrounded by host cell membrane (mCM). In Fig. 2 a mature cell from strain 12.11 (stock culture) has just begun cell division and is approximately 0.5 by 1.25 μm.

Cells maintained in culture show a consistent bacillary form (Fig. 2 and 3), whereas cells growing within host tissues (Fig. 1 and 4) may show irregularity in cell shape, occasionally suggesting the clubbing or terminal swelling that is characteristic of coryneform cells, especially those grown on artificial media (Davis et al. 1973). The tapering from septal to distal end, of cells in the process of cellular division, noted by Barksdale (1970) is seen occasionally.

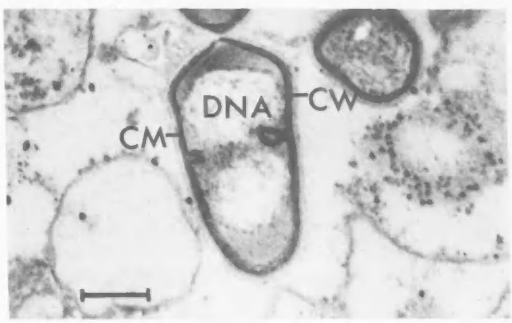

FIG. 4. Experimental infection. Kidney disease bacteria of strain 12.11 located in extracellular region of trout kidney. An early stage of cellular division is shown. The nuclear material (DNA) is disposed as two distinct masses. Cell wall (CW) and cell membrane (CM) are distinct. Bar scale = 0.2 μm.

TABLE 1. Collection schedule and location of bacteria in the kidney, liver, and spleen (K, L, S) specimens from the experimental infection.

Collection day	Specimen number	Bacteria present			
		In specimen	In blood outside organ	Colonizing organ capsule	Within organ
1	1	–	–	–	–
4	2	+	L	–	–
7	3	–	–	–	–
11	4	+	S	–	–
14	5	+	K, L	–	–
18	6	+	K	K, L, S	–
21	7	+	K	L, S	–
25	8	+	–	K	K, L, S
28	9	+	–	K	K, L, S
31	10	+	–	K	K, L, S
33	11	+	–	K	K, L, S
34	12	+	–	K	K, L, S
39	13	+	–	K	K, L, S
41	14	+	–	–	K, L, S
43	15a	+	–	K	K, L, S
	15b	+	–	K	K, L, S
44	16a	+	–	–	K, L, S
	16b	+	–	–	K, L, S
45	17a	–	–	–	–
	17b	+	–	–	K, L, S
46	18a	+	–	–	K, L, S
	18b	–	–	–	–
47	19	–	–	–	–

Both strains of cells feature a similar internal anatomy. The central region of the cells contains lightly staining, filamentous material, generally regarded to represent deoxyribonucleic acid (DNA) (Fig. 1–4). The peripheral region of the cells is filled with small, electron-dense particles that have the size and density characteristics of ribosomes (R). Figure 2 shows a cross section of a cell where a peripheral body, or mesosome (P), extends inward from the cell membrane to the central region of DNA. The cell sectioned longitudinally shows several of these organelles (P) disposed peripherally and indicates why the term "peripheral body" was originally proposed by Chapman and Hillier (1953).

Cell division proceeds in both strains, as it does in bacilli, with the deposition of a transverse cell wall, as originally described in the early ultrathin sectioning studies of *Bacillus cereus* of Chapman and Hillier (1953). (The simultaneous occurrence of membrane septum formation was implied in this work.) This mechanism differs significantly from that in which there is a complete membrane septation before any appreciable deposition of transverse cell wall material occurs (Chapman 1959). In Fig. 2 the cell sectioned longitudinally is in early division and illustrates the initial stages in the partitioning of parental DNA and in the laying down of membrane septum and transverse cell wall material. Figure 3 reveals a cell lying in the left portion of the field that has nearly completed division. Each daughter cell has received a complement of DNA and the membrane septum and transverse cell wall have been almost completed. Peripheral bodies (P), which may appropriately be termed septal mesosomes, appear to connect the DNA of each daughter cell to the transverse septum. The cell on the right in this field shows a completed membrane septum and transverse cell wall. The same mesosomal relationship is apparent. The cellular division process essentially duplicates that described by Kawata (1961) and Barksdale (1970). There is, however, no evidence of the postfission snapping process that is characteristic of many coryneform bacteria (Komagata et al. 1969; Barksdale 1970). It is, of course, possible that the plane of sectioning has been such that this feature was not encountered.

A comparison of the Lamar and 12.11 strains of KD bacteria revealed no significant morphological differences. (Figure 4, which shows a bacterial cell from the experimentally infected tissues, reveals essentially all of the structural features mentioned above.) Furthermore, each of these strains demonstrated the basic cellular components, in essentially the same form, as described by Kawata (1961) and by Barksdale (1970) for *Corynebacterium diphtheriae*: a cell wall (27.0–30.0 nm), somewhat variable in its layering, the exterior surface of which is often quite irregular in form; a cytoplasmic membrane (8.0 nm), presenting the unit membrane configuration (Robertson 1959); numerous ribosomes (10–20 mμ); a nuclear region, filled with

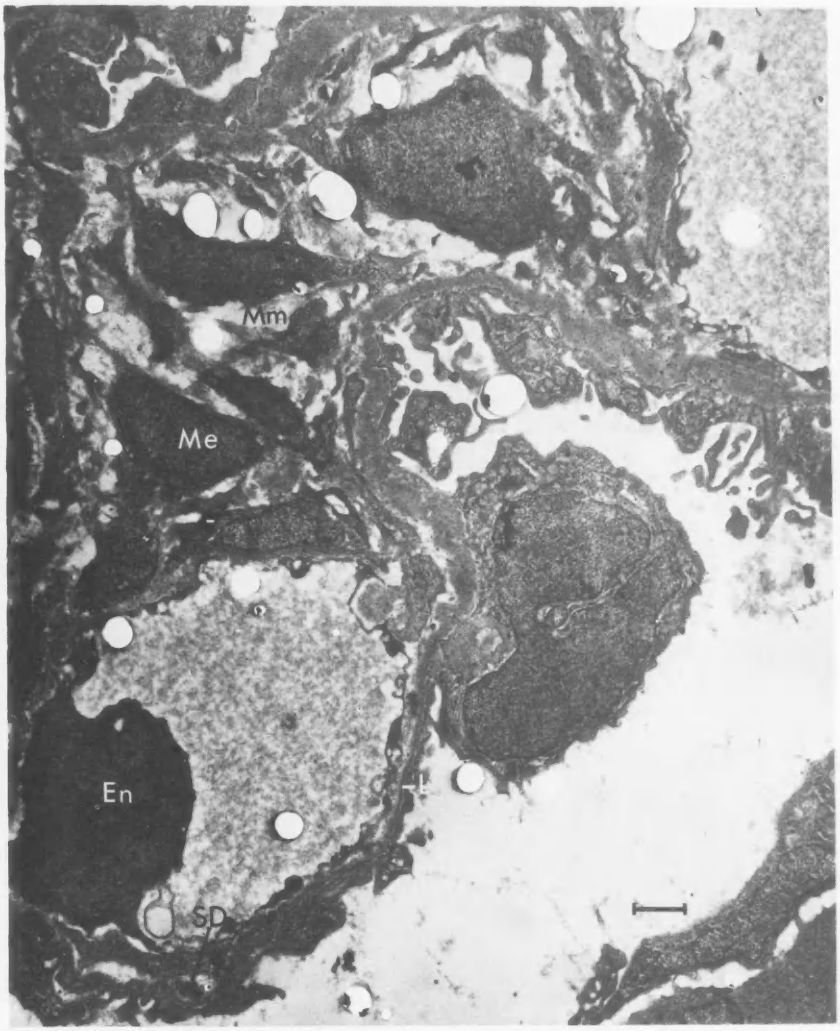

FIG. 5. Experimental infection. Glomerular loop, featuring endothelium, En; lamina densa, L; subendothelial deposit, SD; mesangial cell, Me; mesangial matrix, Mm. Bar scale = 1.0 μm.

fine fibrils (2.0–5.0 nm); an intracytoplasmic membrane system (mesosomes or peripheral bodies), each of which is about 100–500 mμ diam and contains vesicular units (30–40 mμ). (The dimensions given above are those of Kawata [1961].) Neither the present study nor that of Kawata (1961) encountered the electron "opaque" lipid inclusions mentioned by Barksdale (1970), and the present study did not encounter the metachromatic granules mentioned by Kawata (1961) and Barksdale (1970). (Because the lipid inclusions appear as low density areas in the electron micrographs, it would seem more appropriate to describe them as electron lucent.) These differences could be due to differences in culture conditions or to cell ages, as both of these factors influence cellular morphology (Kawata 1961; Barksdale 1970).

As Barksdale (1970) illustrates so effectively with his citations of the differences in cell wall composition between bacteria correctly included in the *Corynebacterium*, *Mycobacterium*, Nocardia (CMN) group, and those (e.g. the plant pathogenic corynebacteria and some propionibacteria) originally but no longer (because of those biochemical differences in wall composition) grouped with the corynebacteria, it is obvious that morphological features of bacteria constitute poor criteria on which to establish taxonomic relationships.

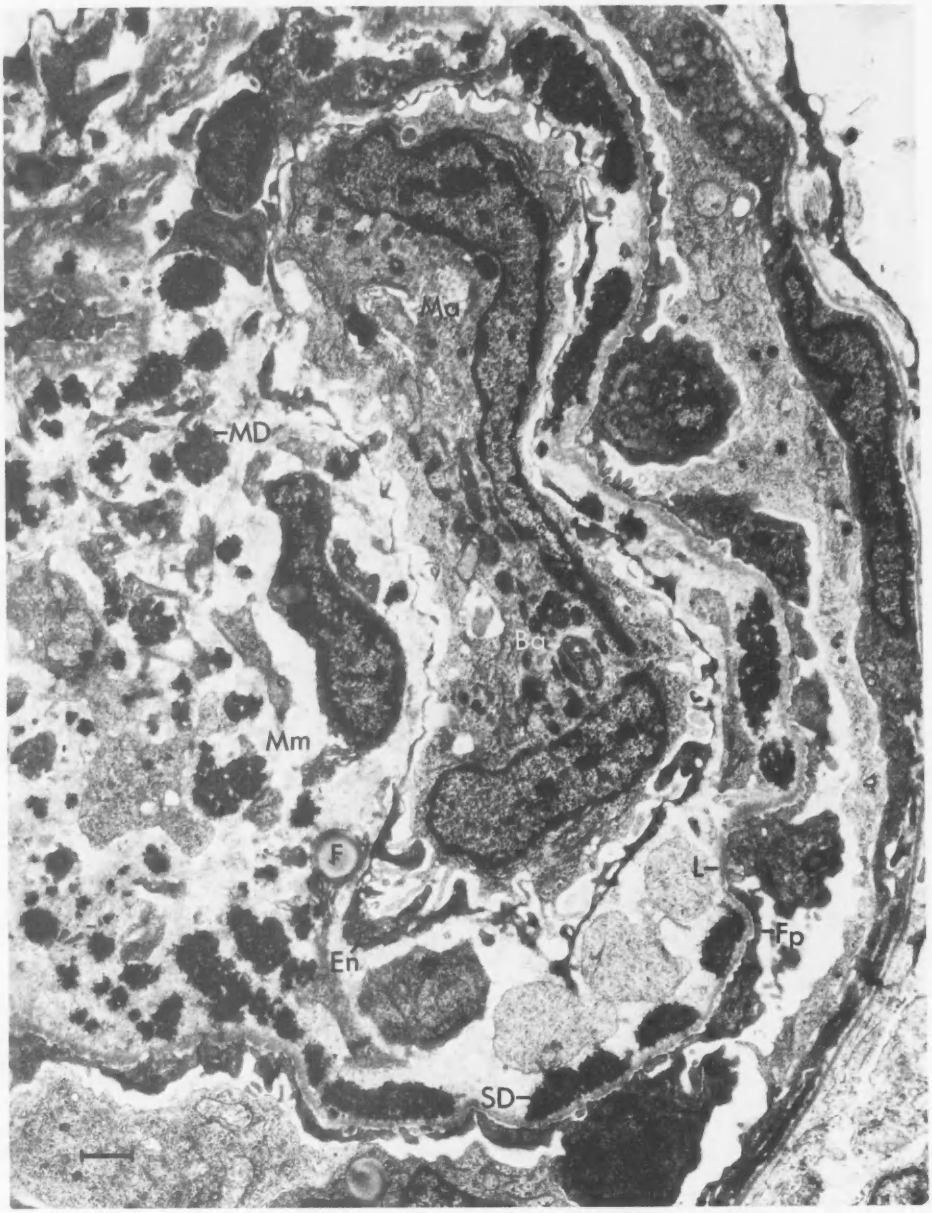

FIG. 6. Experimental infection. Glomerular loop, featuring deposits both subendothelially and mesangially, SD and MD; endothelial cell, En; lamina densa, L; foot processes of visceral epithelium, Fp; fat-like droplet, F. The mesangial matrix, Mm, has expanded, and there is a macrophage, Ma, inside the capillary loop. The macrophage contains a lysosome enclosing a bacterium, Ba. Bar scale = 1.0 μm.

It seems fairly clear, however, that an analysis of cell ultrastructure, including the mode of cellular division, is a worthwhile adjunct to any collection of data being used to determine the classification of bacteria.

THE RENAL HISTOPATHOLOGY

Comparison of the glomerulus of natural and experimental BKD infections revealed similar ultrastructural changes. The same situation occurred in the case of the

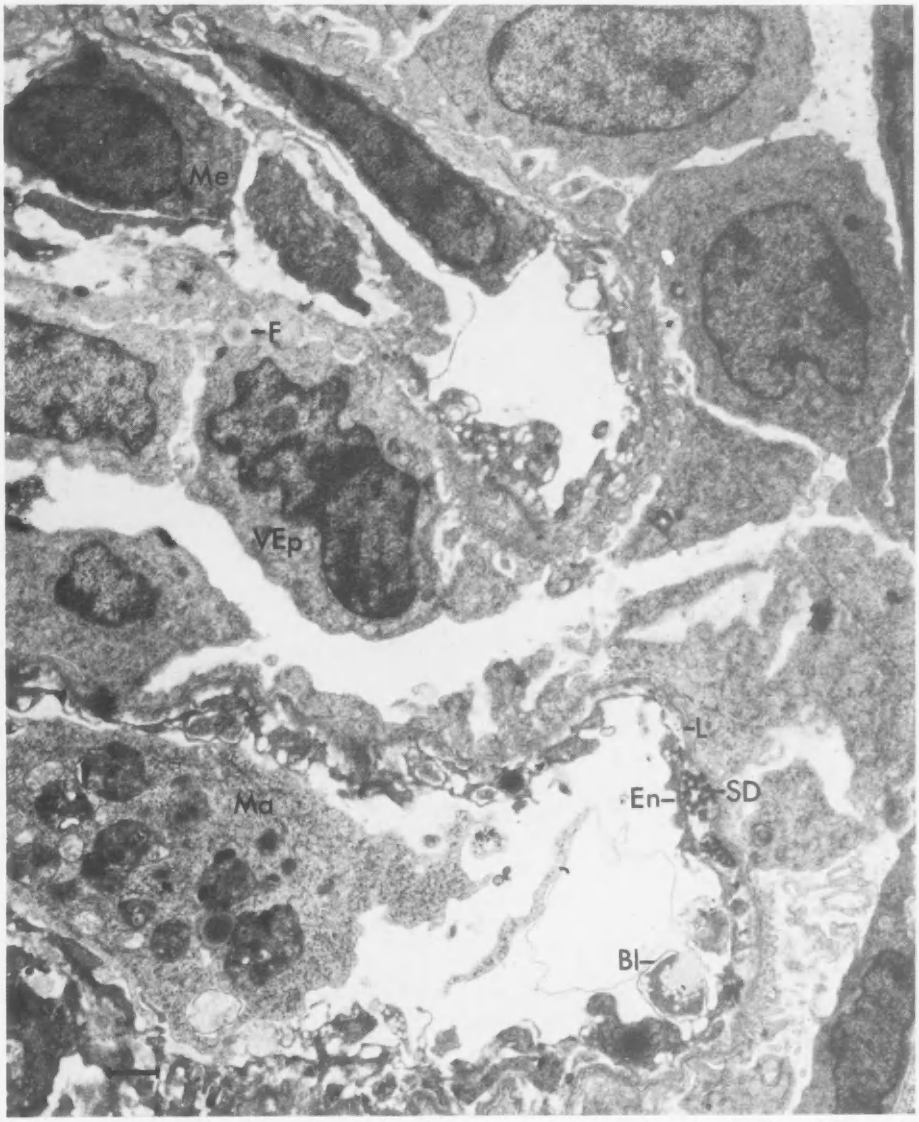

FIG. 7. Natural infection. Two glomerular capillary loops, featuring visceral epithelium, VEp; endothelium, En; bleb, Bl; mesangial cell, Me; fat-like droplet, F; subendothelial deposit, SD; macrophage, Ma; lamina densa, L. Bar scale = 1.0 μm.

kidney tubule. The chronology of these ultrastructural changes was observed uniquely in the experimental infection, where it was possible to examine the kidney at different stages of the disease process. The major histopathological features observed in the experimental infection were substantiated by their additional presence in the fully developed disease of the naturally infected fish.

During the 47 d of specimen collection, 23 experimental fish were killed according to schedule, and 15 fish died spontaneously exhibiting the symptoms of acute BKD. The first spontaneous death occurred on day 31, but 14 such deaths occurred after day 39, prompting an increase in the number of specimens collected after day 41, as is indicated in Table 1. Throughout the experiment, none of the control fish exhibited

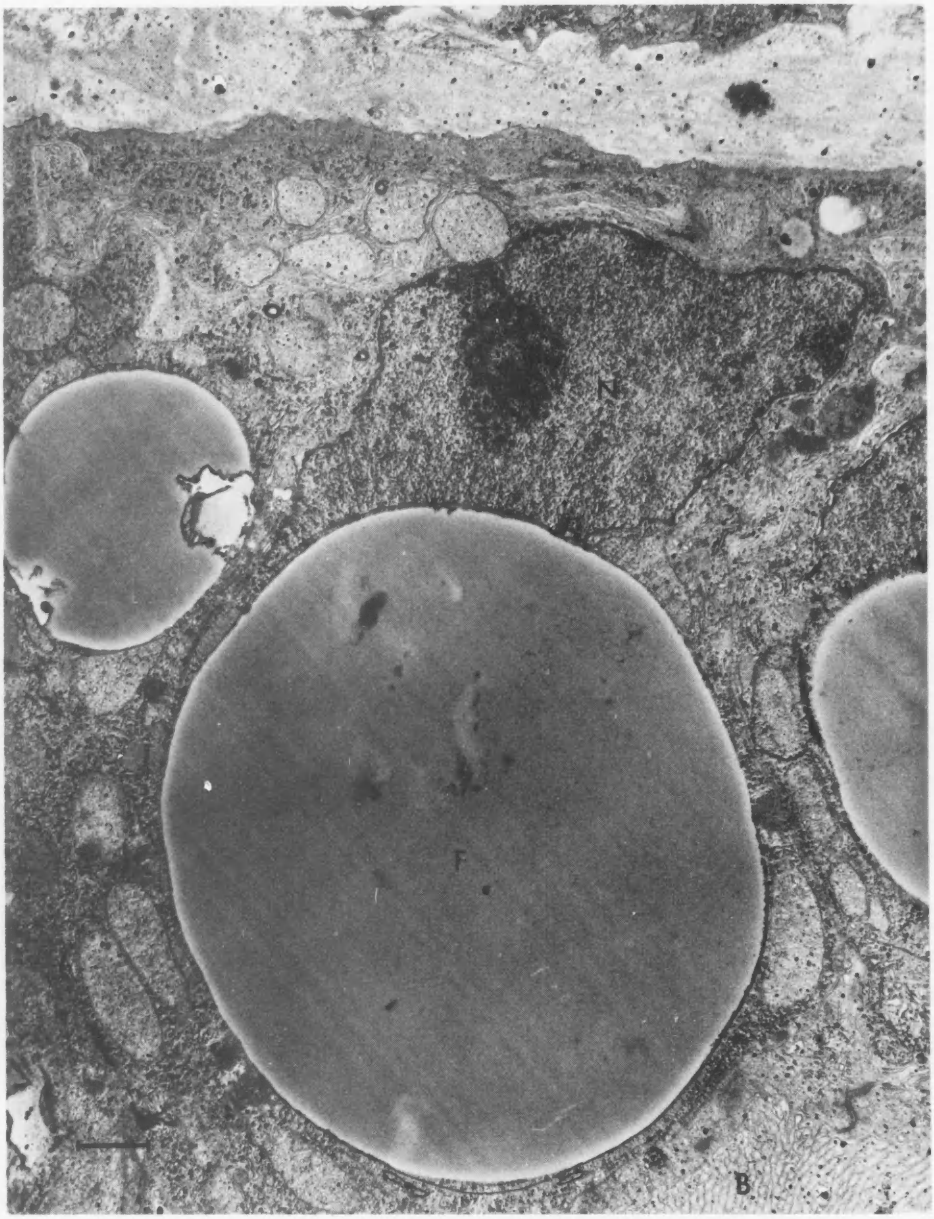

FIG. 8. Experimental infection. Second segment proximal tubule cell from a fish sacrificed on day 41. Cell features include apical brush border, B; nucleus, N; and fat-like droplet, F. Bar scale = 1.0 μm.

disease symptoms, died spontaneously, or carried bacteria in its tissues. Moreover, the fine structure of the control fishes' renal tissues appeared normal at all times and similar to that previously described for brook trout (Anderson and Loewen 1975).

Gram-stained sections from the kidney, liver, and spleen of the experimentally infected fish revealed, as is summarized on a time basis in Table 1, that the bacterial invasion was characterized by, in order of appearance, septicemia, colonization of bacteria on the outer surface of the organs, and penetration of bacteria into deeper lying tissues of the organs. Specimens of the

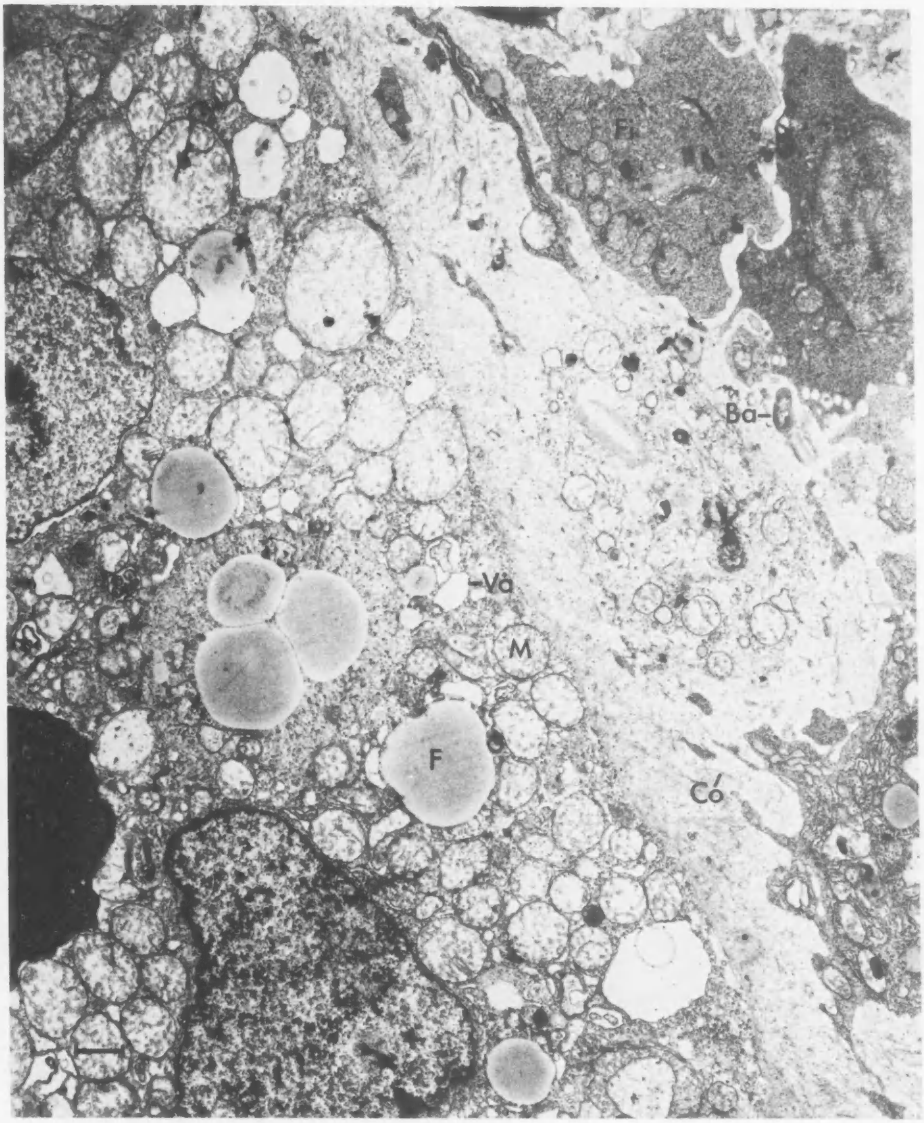

FIG. 9. Natural infection. Basal region of second segment proximal tubule cell, showing fat-like droplet, F; vacuolar space, Va; swollen mitochondria, M. Below the tubule cell is a layer of collagen, Co; free bacteria, Ba, and fibroblasts, Fi. Bar scale = 1.0 μm.

middle region of the longitudinal kidney were chosen for examination on the ultrastructural level. An investigation of the other tissues from the experimental and natural infections awaits completion.

Striking pathological changes in the ultrastructure of the glomerulus occurred in the experimental infection. The earliest change noted was the appearance of a small amount of subendothelial deposits (SD), which were first observed in fish specimen 6, killed on day 18 (Fig. 5). The deposits appeared electron-dense, scanty, and somewhat flocculent. (No glomerular deposits or any other pathological changes were observed in the glomeruli of the control fish kidneys.) As the experimental infection progressed, more severe changes occurred in the glomerulus. For example, deposits occurred both subendothelially (SD) and mesangially (MD) in fish specimen 16b, killed on day 44 (Fig. 6). Here the deposits appeared electron-dense, abundant,

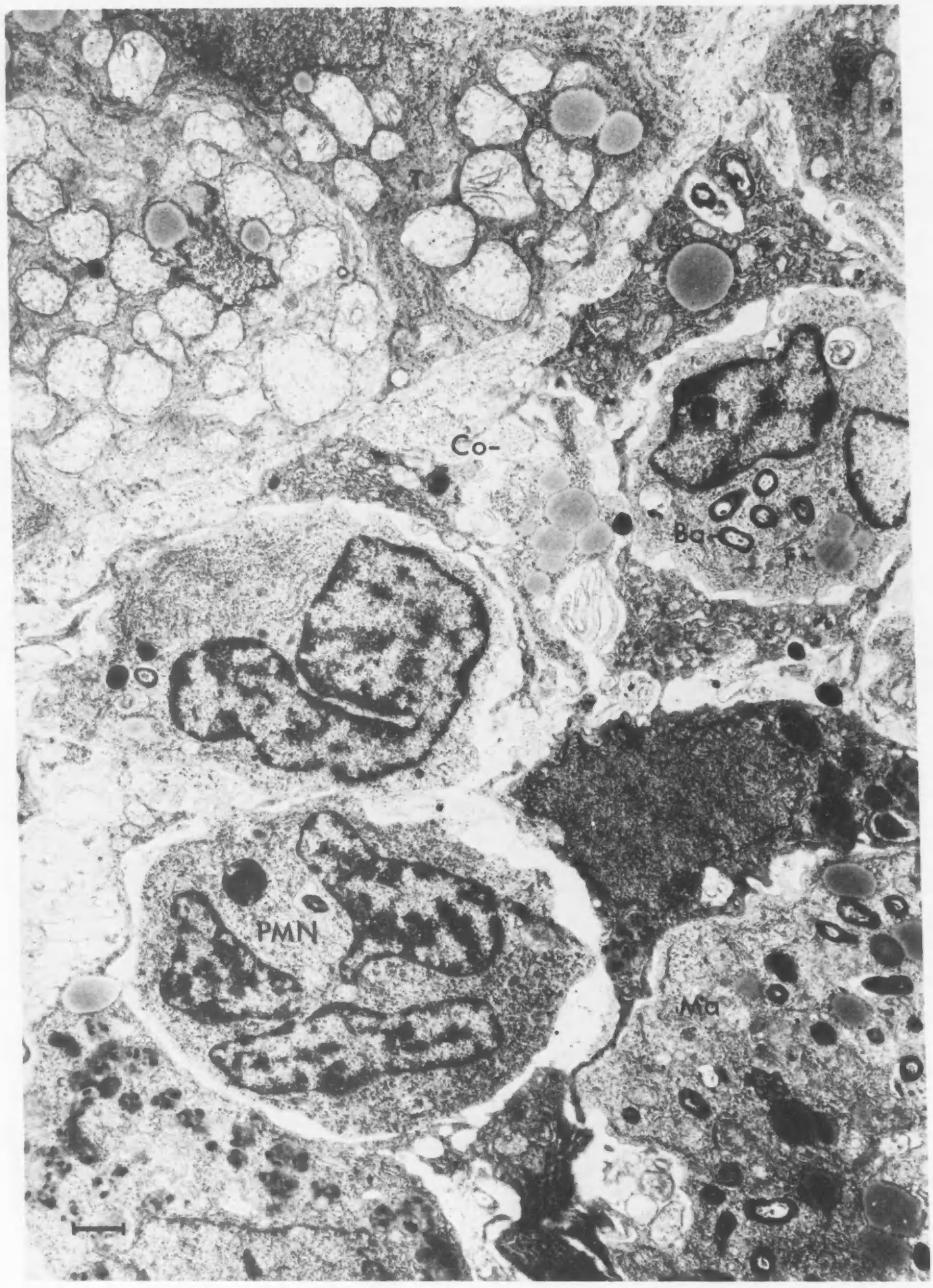

FIG. 10. Natural infection. Cells resembling polymorphonuclear leukocytes, PMN, and macrophages, Ma, appear below a tubule cell, T. Bacteria, Ba, fat-like droplet, F, and collagen, Co, are present. Bar scale = 1.0 μm.

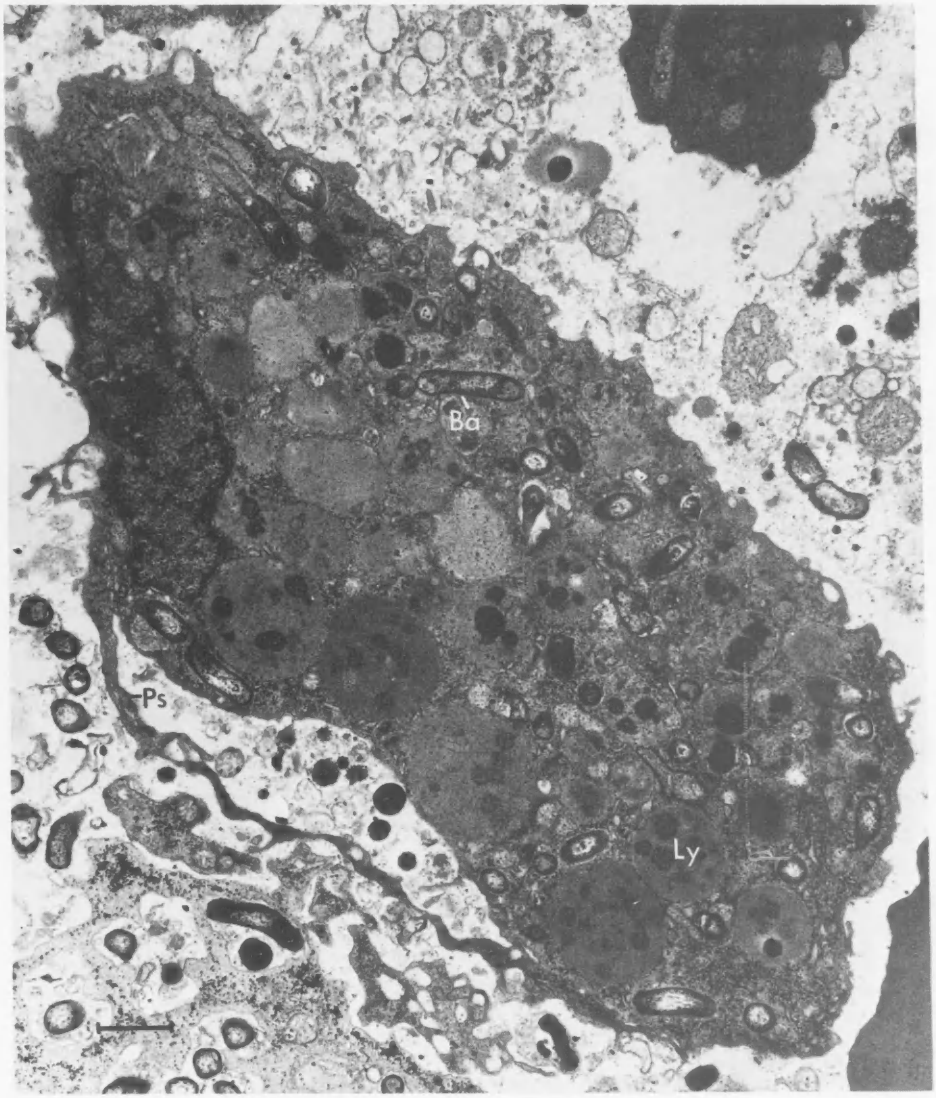

FIG. 11. Experimental infection. Macrophage cell, containing bacteria, Ba, secondary lysosomes, Ly, and pseudopodic arm of cytoplasm, Ps. Bar scale = 1.0 μm.

and somewhat granular. Also, the mesangial matrix had proliferated and fat-like droplets occurred in endothelial cells and in visceral and parietal epithelial cells. The endothelial lining of the capillary loops contained gaps and some of the foot processes of the visceral epithelial cells appeared fused. The basement membrane, or lamina densa, appeared slightly thickened and mottled.

The majority of these glomerular changes occurred also in the naturally infected fish (Fig. 7). Similar subendothelial deposits and endothelial and epithelial fat-like droplets were present. Large, structureless blebs (Bl) projected from the endothelial cells into the capillary lumen, indicating diffuse edema in these cells. Glomerular changes of these kinds have been observed in mammalian renal disease. Edematous endothelial

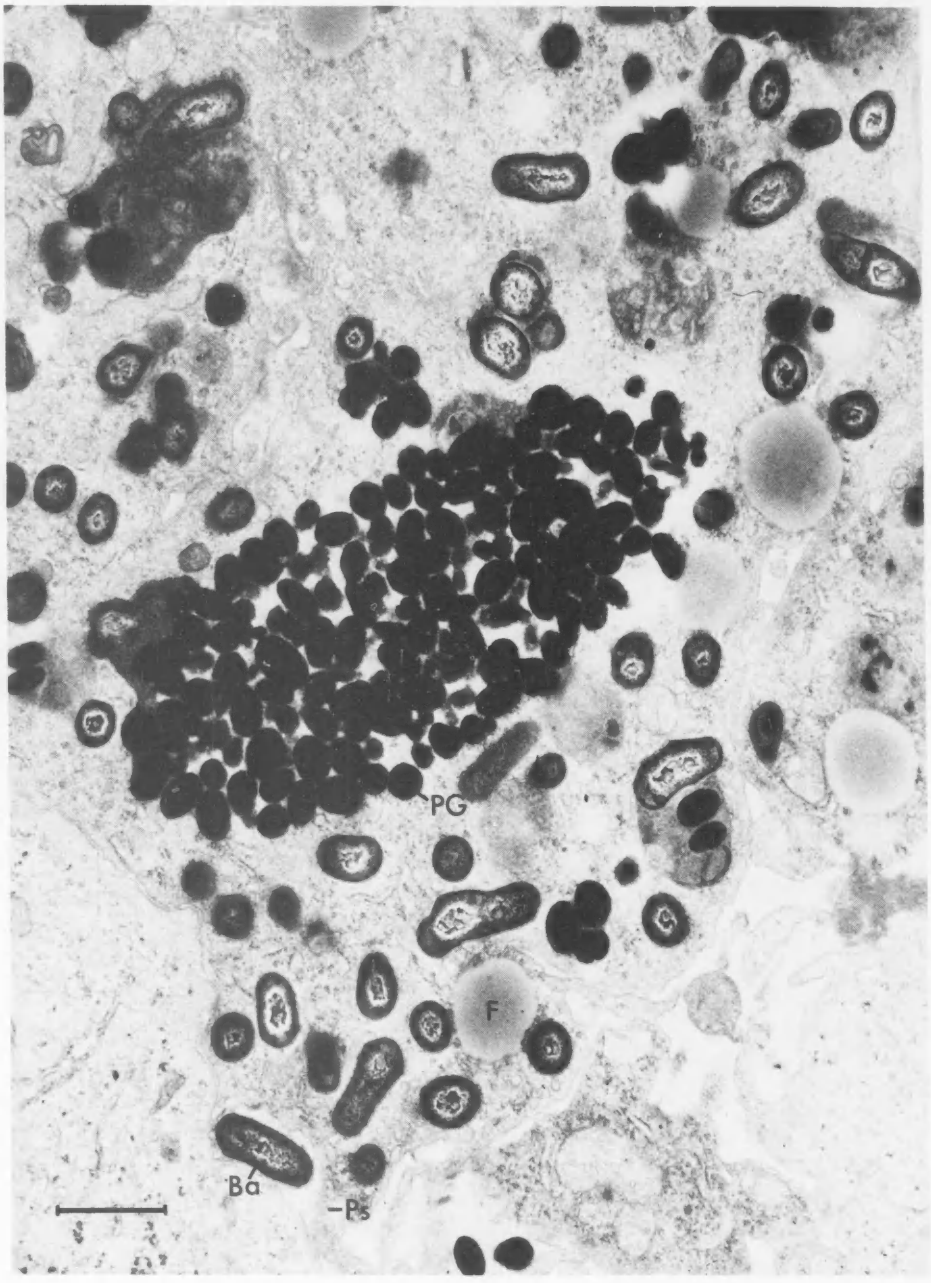

FIG. 12. Natural infection. Pigment cell contains pigment granules, PG, and fat-like droplets. F. The cell has pseudopodic extensions of cytoplasm, Ps, and appears to be phagocytosing bacteria, Ba. Bar scale = 1.0 μm.

cells occlude the lumen in acute glomerulonephritis, and fusion of the foot processes of the visceral epithelial cells is encountered in nearly all glomerular diseases, but most prominently in severe proteinuria and nephrotic syndrome (Churg 1968). The subendothelial deposits resemble antigen–antibody complexes that accumulate in nephrotoxic serum nephritis (King 1966; Fish et al. 1966). Thickening and irregularity of the basement membrane occur in various diseases including glomerulonephritis and are usually accompanied by proteinuria (Churg 1968). Proliferation of the mesangium is characteristic of both acute renal inflammation and chronic renal disease such as that associated with diabetes (Churg 1968).

The earliest ultrastructural evidence of damage in the renal tubules of the experimental infection was hydropic change. Proximal and distal tubule cells from fish specimen 6, killed on day 18, featured swollen mitochondria and a dilated endoplasmic reticulum. The light microscope revealed that cells of the first proximal segment in fish specimen 9, killed on day 28, had accumulated large, eosinophilic apical granules closely resembling hyaline granules. As the infection progressed, fat-like droplets (F) accumulated in the cytoplasm of all of the other segments of the tubule, occasionally to a striking degree (Fig. 8), indicating fatty change in the cells. Alterations characteristic of irreversible cell injury, such as ruptured mitochondria, changes in lysosomes and nuclear morphology, and disruption of both the brush border and the plasmalemma's basal infoldings, were observed more frequently towards the end of the experiment, but as early as day 31 in the proximal segment cells of fish specimen 10.

Two of the changes observed in the experimental infection, viz. fatty change and swollen or ruptured mitochondria, occurred frequently in the tubule cells of the well-developed natural infection. Although the second proximal segment of the normal trout nephron has been reported to contain fat droplets (Anderson and Loewen 1975), the second proximal segment in the Lamar fish revealed an unusually large amount of fat-like droplets (Fig. 9). The ruptured mitochondria and multiple vacuolar spaces adjacent to the endoplasmic reticulum of these cells suggest that irreversible cell injury also was occurring.

Evidence of a host immune and inflammatory response was present in both the experimental and natural infections. Host cells resembling polymorphonuclear leucocytes, macrophages, and pigment cells phagocytosed bacteria (Fig. 10–12). The morphological integrity and active cell division of the phagocytosed bacteria indicate that the bacteria were not necessarily being killed by the phagocytic cells. Collagen was deposited at the basal border of proximal and distal tubule cells in both infections, possibly in response to tubule cell injury. Stellate cells resembling fibroblasts, which contain a well-developed granular endoplasmic reticulum and Golgi apparatus, appeared immediately next to the collagen deposits (Fig. 9). In the experimental infection, fibroblasts and other cells of the interstitial tissue of the kidney accumulated fat-like droplets.

The pathological changes of the renal tubule noted above resemble those found in mammalian proteinuria and glomerulonephritis. Dilated tubule cells are found in human chronic pyelonephritis resulting from bacterial infection (Dunnill 1976). Hyaline granules probably consist in part of protein resorbed from the glomerular filtrate in cases of proteinuria (Robbins 1974) and have been observed in mammalian glomerulonephritis (Netter 1973). Bendele and Klontz (1975) described hyaline droplets in the kidney of rainbow trout experimentally infected with kidney disease bacteria, and Hunn (1964) noted a 52% reduction in total plasma protein in yearling trout infected with chronic kidney disease. Fatty degeneration is a sign of acute cell injury in human renal disease and is present in most cases of nephrotic syndrome (Meadows 1973).

In summary, pathological changes in the fine structure of both the glomerulus and renal tubule were present in BKD, and these changes resembled those observed in such mammalian renal diseases as glomerulonephritis and nephrotic syndrome. The evidence for these proposed histopathological events is morphological and should be supported by further physiological and histochemical studies.

Acknowledgments

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Production, Mortality, and Sustainable Yield of Northwest Atlantic Harp Seals (*Pagophilus groenlandicus*)

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WINTERS, G. H. 1978. Production, mortality, and sustainable yield of Northwest Atlantic harp seals (*Pagophilus groenlandicus*). J. Fish. Res. Board Can. 35: 1249–1261.

From recent and historical data the natural mortality rate of adult harp seals (*Pagophilus groenlandicus*) is estimated to be 0.10 which is within the range of previous estimates (0.08–0.11). New estimates of bedlamer and 0-group natural mortality rates were not significantly different from those of adult seals. Pup production estimates from survival indices agreed well with those from sequential population analyses and indicated a decline from about 350 000 animals in the early 1950s to about 310 000 animals in the early 1970s. Over the same period the 1+ population size declined from 2.5 to 1.1 million animals but has been increasing at the rate of 3%/yr since the introduction of quotas in 1972. The relative contribution of the "Front" production to total ("Front" plus Gulf) production during the past decade has fluctuated from 49 to 87%, the average of 64% being very similar to the 61% obtained previously. These fluctuations suggest some interchange between "Front" and Gulf adults and it is concluded that homing in the breeding areas is a facultative rather than obligatory aspect of seal behavior. Thus the heavier exploitation of the "Front" production is probably sufficiently diffused into the total population to avoid serious effects on "Front" production. The maximum sustainable yield of Northwest Atlantic seals harvested according to recent patterns is estimated to be 290 000 animals (80% pups) from a 1+ population size of 1.8 million animals producing 460 000 pups annually. The sustainable yield at present levels of pup production (335 000 animals) is calculated to be 220 000 animals which is substantially above the present TAC of 180 000 animals and coincides with present harvesting strategies designed to enable the seal hunt to increase slowly towards the MSY level.

Key words: mortality, production, sustainable yield, population dynamics, marine mammal

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En se basant sur des données récentes et passées, on estime que le taux de mortalité naturelle des phoques du Groenland adultes est de 0.10, ce qui est dans les limites des estimations antérieures (0.08–0.11). Les nouvelles estimations des taux de mortalité naturelle des brasseurs et des individus d'âge 0 ne diffèrent pas de façon significative de celles des phoques adultes. Les estimations de production de chiots à partir d'indices de survie s'accordent bien avec celles d'analyses séquentielles de populations et indiquent un déclin, passant de plus de 350 000 animaux au début des années 1950 à plus de 310 000 animaux au début des années 1970. Durant la même période, la population d'âge 1+ passa de 2.5 millions à 1.1 million d'individus, mais avec l'introduction des contingents en 1972, elle a augmenté au rythme de 3%/a. La contribution relative du Front à la production totale (Front plus Golfe) durant la dernière décennie a varié entre 49% et 87%, la moyenne de 64% se rapprochant de 61%, moyenne obtenue antérieurement. Ces fluctuations nous donnent à penser qu'il y a échange entre les adultes du Front et ceux du Golfe, et nous concluons que le « homing » sur les lieux de reproduction est un aspect facultatif plutôt qu'obligatoire du comportement social. Une chasse plus intensive sur le Front est probablement suffisamment diffuse parmi la population totale pour éviter de sérieux effets sur la production du Front. On estime que la rendement maximum soutenu des phoques de l'Atlantique Nord-Ouest, avec les régimes de chasse des dernières années, est de 290 000 animaux (80% étant des chiots) à partir d'une population d'animaux d'âge 1+ de 1.8 million produisant 460 000 chiots annuellement. On calcule que le rendement soutenu aux niveaux actuels de production de chiots (350 000) est de 220 000 animaux, ce qui est beaucoup plus que le total des prises admissibles de 180 000 animaux et qui coïncide également avec les stratégies actuelles de récolte conçues de façon que le nombre d'individus tués augmente graduellement pour atteindre le rendement maximum soutenu.

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COMMERCIAL exploitation of the harp seal population of the Northwest Atlantic began late in the 18th century when the demand for skins in the luxury clothing trade initiated the development of a large fleet of schooners capable of harvesting the "whelping" (breeding) herds in the "Front" area off northern Newfoundland. Harvest levels by vessels operating on the "Front" increased rapidly from 80 000 pelts in 1805 (Chafe et al. 1923) to an average of 225 000 pelts during the period 1817–26; from 1830 to 1850 the average offshore kill exceeded 450 000 pelts including a peak harvest of 687 000 pelts in 1844. The average harvest remained around 450 000 pelts until the 1860s when it dropped to about 310 000 pelts, remaining at that level until World War I. During the 1920s and 1930s the hunt produced an average harvest of 150 000 pelts declining to about 35 000 pelts during World War II. From 1946 to 1971, the annual harvest (Front and Gulf including landsmen) increased to an average of 300 000 animals but has averaged about half that level since the introduction of quotas in 1972. Thus over the past 150 yr the average (minimum) annual kill of harp seals has been about 275 000 animals.

Recent analyses of the status of the Northwest Atlantic harp seals have provided conflicting estimates of current and historical population levels as well as wide ranging estimates of mortality rates and sustainable yields. As a result of such differences advice on required conservation measures have ranged from a substantial reduction in the harp seal hunt based mainly on the results of aerial surveys (Lavigne 1976) and projections of pup production and survival (Ricker 1975) to provisions for increasing the annual kill based mainly on analytical population models (Benjaminsen and Øritsland 1975a; Lett and Benjaminsen 1977). Such discrepancies have been mainly due to incomplete data sets, variability in catch data, and inherent biases due to changes in hunting patterns and pup production. Since parameters of mortality and reproductive potential are critical to prognoses of sustainable kill and population change, a reanalysis of most recent data has been carried out and the results are documented below.

Materials and Methods

Age composition data have been obtained from Benjaminsen and Øritsland (1975a), Sergeant (1977a), and as summarized and amalgamated into catch-at-age data by Lett and Benjaminsen (1977). Catch and effort data for the various components of the seal harvest have been obtained from annual issues of the ICNAF Statistical Bulletin series.

Pup production and population size have been calculated by two methods, the Survival Index Method (SI) and a method of sequential computation developed by Pope (1972). The Survival Index Method is essentially that described by Sergeant (1971) as modified by Benjaminsen and Øritsland (1975b) and involves relating survival indices of successive year-classes to the number of pups killed of each year-class. In this analysis survival index is defined as

$$\text{SI} = \frac{\text{Age-group frequency as percent of annual catch}}{\text{Total age-group frequency as percent of total catch}}$$

Estimates of survival index by this method may be biased because equivalent pup kills may produce different survival rates, depending on the level of pup production in a particular year. However, since harp seal populations can only undergo slow oscillations in size (Sergeant 1976a), selection of a narrow time series should substantially reduce such biases.

The cohort method of sequential computation developed by Pope (1972) assumes some seasonal variation in the pattern of natural and hunting mortality which is characteristic of the seal fishery. The method is based on the approximate formula

$$N_i = C_i e^{M/2} + N_{i+1} e^{M}$$

where N_i and C_i are age-specific population size and catch of a cohort, respectively, and M is the instantaneous coefficient of natural mortality. The model is initiated by an estimate of population size of a cohort in its most recent year in the fishery (N_{i+1}) using the following equation (Baranov 1918):

$$N_{i+1} = \frac{C_{i+1}(F_{i+1} + M)}{F_{i+1}(1 - e^{F_{i+1}+M})}$$

where F_{i+1} is the hunting mortality in the most recent year. Hunting mortality is most commonly calculated as a direct function of hunting effort.

Results

Estimation of Pup Production from Survival Indices

To avoid smoothing effects arising from possible errors in age reading, survival indices have been calculated for ages 8 and younger, excluding age-group 1 because of variability in the selectivity of this age-group. Age composition data pertain only to large vessel samples obtained from the "Front" (Lett and Benjaminsen 1977). Mean survival indices are given by year-class in Table 1 and these have been related to pup kill by two-way regression analyses using an 8-yr time series from which a functional estimate (Ricker 1973) of median pup production has been derived. The results (Table 1) indicate that pup production (i.e. effective breeding stock) declined from a level of 500 000–535 000 animals in the early 1950s to 320 000 animals in 1972. However, the decline in pup production has been relatively modest since the early 1960s, reflecting perhaps the effect of regulations introduced in 1961 (and progressively strengthened since then) restricting the duration of the hunting season and also reducing the kill of breeding females.

Estimation of Mortality Rates of Adult Seals

Estimates of total (Z) and natural (M) mortality rates (Table 2) were derived from analyses of catch curves based on age composition data of molting male harp seals for the years 1969–74 from Benjaminsen and Øritsland (1975a). Age-groups 5–17 only were considered in mortality estimates since these age-groups not only defined the major portion of the adult population but also coincided with the range of year-classes

TABLE 1. Survival indices and estimated pup production of harp seals for median year 1952–72. Survival indices (weighted by age) are calculated from age composition data of large vessel kills on the "Front."

Year-class	Age range	Mean survival index (Y)	Pup kill ('000) (X)	Year-classes	Median year pup production $R_{y \cdot x}$	$R_{x \cdot y}$	Geometric mean pup production	Correlation coefficient
1948	4–7	0.82	137					
1949	3–6	0.73	227					
1950	2–8	0.87	226					
1951	2–7	0.71	302					
1952	2–6	1.14	198	1948–56	430	666	535	.68
1953	2–8	0.90	198	1949–57	463	530	495	.88
1954	4–8	1.11	185	1950–58	505	549	527	.94
1955	3–8	0.84	260	1951–59	477	572	522	.86
1956	2–8	0.56	347	1952–60	413	531	468	.79
1957	4–8	1.07	172	1953–61	415	528	468	.80
1958	3–8	1.12	149	1954–62	393	517	451	.77
1959	2–8	1.15	243	1955–63	397	495	443	.80
1960	2–8	1.48	164	1956–64	398	492	443	.80
1961	2–8	1.21	175	1957–65	338	483	404	.69
1962	2–8	0.74	211	1958–66	346	466	402	.72
1963	2–8	0.66	286	1959–67	347	423	383	.78
1964	2–8	0.78	271	1960–68	339	380	359	.86
1965	2–8	0.92	187	1961–69	339	397	366	.81
1966	2–8	0.79	255	1962–70	325	396	359	.75
1967	2–8	0.62	280	1963–71	334	393	362	.80
1968	2–8	1.65	160	1964–72	342	406	372	.82
1969	2–8	0.98	237	1965–73	342	390	365	.88
1970	2–7	0.71	220	1966–74	350	381	365	.92
1971	2–6	0.91	213	1968–74	322	379	349	.88
1972	2–5	1.41	120	1970–74	315	325	320	.98
1973	2–4	1.62	103					
1974	2–3	1.68	118					

TABLE 2. Calculation of mortality rates of molting harp seals (adult males) from catch curve analyses.

Year-classes	Sampling year	Slope of catch curve (A)	Instant. ΔR (B)	Instant. ΔF (C)	$\bar{Z}$ (D)	F (E)	M (F)
1952–64	1969	−.061	−.083	−.015	.159	.058	.101
1954–66	1971	−.075	−.080	−.006	.161	.060	.101
1956–68	1973	−.126	−.059	+.025	.160	.051	.109
1957–69	1974	−.085	−.080	+.035	.130	.050	.080
Mean (± SE)		−.087	−.076	+.010	.153	.055	.098±0.011

for which estimates of pup production were available from survival indices. Pup production over the period 1952–69 has been declining (Table 1) and this will have the effect of biasing downwards estimates of mortality from catch curves. To adjust for such biases, instantaneous rates of change in recruitment (Ricker 1975) have been calculated for the various year-classes included in the annual catch curve (Table 2).

Changes in hunting mortality (F) with time will also producee a bias in mortality estimates from catch curves. One measure of hunting mortality is exploitation rate (μ) which is approximately equal to F at values of $\mu \leq 0.10$. Estimates of pup production (Table 1) have been converted to equivalent female breeding stock size by dividing by the fertility rate (Sergeant 1966, 1969, 1970). The adult male population size for each year was then calculated from population sex ratio data given in Lett and Benjaminsen (1977) thus allowing computation of instantaneous exploitation rates from data on the sex composition of the kill (Lett and Benjaminsen 1977). These exploitation rates (Table 3) were accumulated for each year-class up to (but not including) the sampling year and were then divided by the number of years (ages) involved to produce an estimate of mean hunting mortality exerted on each year-class prior to the year of catch curve analysis. The slopes of the regression lines of average hunting mortality (i.e. μ) (log transformed) against age for each sampling year were then calculated (Table 2).

TABLE 3. Calculation of the instantaneous exploitation rates of adult male and female harp seals from data on sex ratio of the kill and the breeding population. Estimates of female breeding stock (divided by the fertility rate) are derived from the Survival Index Method.

Year	Total adult kill ('000)	Adult kill sex ratio (% males)	Female breeding stock ('000)	Adult sex ratio (% males)	Male breeding stock ('000)	Instant. exploit. rate	
						Males	Females
1952	79.2	50	563	50.0	563	.073	.073
1953	38.0	50	521	50.0	521	.038	.038
1954	38.8	50	555	50.0	555	.036	.036
1955	37.7	50	549	50.0	549	.036	.036
1956	31.3	50	493	50.0	493	.032	.032
1957	47.8	50	493	50.0	493	.050	.050
1958	103.0	50	475	50.0	475	.115	.115
1959	41.6	50	466	50.0	466	.046	.046
1960	48.5	50	466	50.0	466	.054	.054
1961	7.9	50	425	50.0	425	.009	.009
1962	28.0	54	423	50.0	423	.036	.031
1963	52.0	54	403	49.8	399	.074	.061
1964	54.6	62	378	49.2	366	.097	.056
1965	26.0	63	386	48.4	362	.046	.025
1966	38.0	71	378	48.1	350	.080	.030
1967	35.4	68	381	47.2	341	.071	.030
1968	22.3	71	392	46.6	342	.047	.017
1969	26.0	79	384	45.9	326	.065	.014
1970	18.6	74	384	45.1	316	.045	.013
1971	8.8	75	367	45.1	322	.021	.006
1072	8.4	55	350	45.5	292	.016	.011
1973	16.6	71	350	46.2	301	.040	.014

Estimates of total instantaneous mortality rates adjusted for changes in recruitment and hunting mortality are shown in Table 2. These reflect the mortality characteristics of the adult male population during the 1960s and 1970s. Subtraction of hunting mortalities (as calculated above) from these values of $\bar{Z}$ provides estimates of the instantaneous natural mortality rate (M) (Table 2). The average natural mortality rate of adult males calculated in this manner (0.098 ± 0.011) may be compared with other estimates of M of 0.114 (Lett and Benjaminsen 1977), 0.106 (Benjaminsen and Øritsland 1976) and 0.08 (Ricker 1971).

Prior to the 1960s the seal hunt was essentially unregulated and undoubtedly the reported statistics were incomplete both as a result of an inadequate collection system and significant pelt losses in the absence of regulations requiring daily kills to be accounted for in the reported catch. These losses will be evident in catch curves as extra mortality. Mortality rates have been calculated for three year-classes (1947–49) exploited during the 1950s from catch-at-age data given in Lett and Benjaminsen (1977). Although sampling data during this period are admittedly poor, the results (Table 4) indicate that a natural mortality rate of 0.12 probably reflects the increased mortality during the 1950s due to kill losses and incomplete reporting.

TABLE 4. Calculation of mortality rates of harp seals from analyses of catch curves for the 1950s. Effort data are derived from Norwegian sealing statistics as reported in the ICNAF Statistical Bulletin Series.

	Year-class			
	1947	1948	1949	Combined
Age range	5–11	4–10	3–9	
Slope	.024	.004	.021	.017
Instant. Δ effort	.143	.143	.143	.143
$\bar{Z}$	.167	.148	.164	.160
F				.040
M				.120

ESTIMATION OF BEDLAMER MORTALITY RATES

Mortality rates of bedlamer (immature) harp seals from age-groups 1 to 3 have been calculated by the linear formula of Paloheimo (1961) using catch-at-age data (Lett and Benjaminsen 1977) for the period 1966–71 when the age-specific selectivity pattern was fairly stable. Selection factors have been calculated from age-specific hunting mortalities as derived from cohort analyses (see later section) and effort data are those given in Table 7. The results (Table 5) indicate $\bar{Z} = 0.166$ and $\bar{Z} = 0.160$ for age-groups 1–2 and 2–3, respectively. Estimates of total mortality ($\bar{Z}$) for these age-groups from cohort analyses $\bar{Z}_c$ ($M = 0.10$) for the same period (Table 5) are very similar in magnitude, suggesting that the natural mortality rate of bedlamer seals is not substantially different from that of adult seals.

The natural mortality rate of 0-group harp seals can

TABLE 5. Calculation of total mortality rates (Z) of bedlamer harp seals by the method of Paloheimo (1961) using catch-at-age data for the period 1966–71. Selection factor is defined as the ratio F/F_3+ as determined from cohort analyses.

Age	Average kill ('000) A	Selection factor B	Adjusted kill (A/B) C	Hunting effort D	Kill per unit effort (C/D) E	Z	Z_c
1	12.37	2.22	5.57	14.20	.392	.166	.175
2	5.04	1.25	4.03	12.15	.332	.160	.150
3	2.87	1.00	2.87	10.13	.283		

TABLE 6. Forward projection of harp seal production and population size from 1972 to 1978 according to the assumptions listed in the text.

Year/Age	Population size ('000) at age 0	1	2	3	4	5	6	7+	1+ Population size
1972	320	123	111	86	118	40	58	555	1091
1973	322	181	110	99	76	105	35	548	1154
1974	315	198	161	96	87	67	91	512	1212
1975	313	179	170	141	85	77	59	532	1243
1976	310	157	153	148	124	75	68	523	1248
1977	321	161	132	132	130	110	66	526	1257
1978	335	176	140	115	115	114	98	526	1284

be obtained from estimates of the number of residual pups (i.e. those surviving the spring pup kill) in relation to the number of age-group 1 seals. Estimates of the number of residual pups are available from pup production estimates by the Survival Index Method minus the pup harvest; these are compared below with numbers of 1-yr-olds estimated by cohort analyses (Table 8) for the year-classes 1961–70 ($M = 0.10$):

Avg pup prod.	Avg pup kill	No. residual pups (N_0)	No. age 1 (N_1)	$\log_e N_0/N_1$ (Z)
374 000	220 000	154 000	141 000	.088

The analyses suggest that the natural mortality rate of young harp seals during their 1st yr of life is not significantly different from that of older age-groups.

CALCULATION OF PUP PRODUCTION 1973–78

Estimates of pup production from survival indices are also estimates of the effective breeding stock (i.e. successful pregnant females). With a method devised by Ricker (1971) and modified as below, escapements from the 1966 to 1971 year-classes have been projected to 1972 (the last year for which an estimate of pup production is available) under the following assumptions:

1) a maturity ogive as given in Sergeant (1976b),

2) a pregnancy rate of 0.92 (Sergeant 1966, 1969, 1970),

3) sex ratio and catch-at-age data as given in Lett and Benjaminsen (1977),

4) a pup production of 320 000 animals in 1972.

By subtracting the catch, 1966–71 escapements were brought forward to 1972 ($M = 0.10$) to represent the population aged 6–1, respectively. After applying the pregnancy rate, maturity ogive, and sex ratio data to these age-groups, the remaining animals required to make up the breeding stock were calculated by subtracting the sum of the breeding females aged 1–6 from the 1972 pup production. From these calculations the total 1+ population may also be calculated and brought forward to 1978 in a similar manner as described above. The results (Table 6) suggest a gradual decline in pup production to 310 000 animals in 1976 reflecting the heavy pup kills in the 1966–71 period but a substantial resurgence in pup production to 335 000 animals in 1978 reflecting the increased survival of year-class 1972 onwards as a result of quota impositions. The 1+ population size has steadily increased from approximately 1.1 million animals in 1972 to almost 1.3 million animals in 1978 representing an instantaneous rate of increase of 0.03.

TRENDS IN HUNTING EFFORT 1966–76

Extensive hunting effort statistics are available for the Norwegian sealing fleet from the ICNAF Statistical Bulletin series and these have been compiled for the period following the protection of breeding females in 1965. Before then the hunting preferences of the Norwegian fleet at the "Front" were directed largely to-

TABLE 7. Calculation of hunting effort on the basis of kill statistics of the Norwegian sealing fleet.

Year	Norwegian fleet			Total Norwegian effort	Total kill		1+ Kill	1+ Effort
	No. men	No. days hunting	Avg HP ('000)		Norwegian kill	Total effort	total kill	
	(*A*)	(*B*)	(*C*)	(*D*)	(*E*)	(*F*)	(*G*)	(*H*)
1966	403	74	1.25	37288	1.99	74203	0.25	18550
1967	465	70	1.31	42641	1.81	77180	0.17	13120
1968	240	55	1.38	18216	2.27	41350	0.20	8270
1969	364	66	1.39	33393	2.51	83815	0.20	16765
1970	325	58	1.50	28275	2.29	64750	0.16	10400
1971	220	54	1.57	18652	2.41	44950	0.10	4495
1972	209	52	1.67	18150	2.56	46464	0.12	5575
1973	180	56	1.63	16430	2.29	37625	0.22	8300
1974	146	56	1.65	13490	2.77	37367	0.23	8600
1975	149	48	1.73	12373	2.94	36375	0.20	7300
1976	113	41	1.92	8895	3.88	34513	0.20	6905

wards bedlamers and adults which constituted nearly 50% of the kills in the period 1950–65 compared with <20% in the period 1966–76. This change in hunting pattern does not allow a direct comparison of effort statistics in the two periods and hence only the period 1966–76 has been selected.

Hunting effort by the Norwegian fleet was calculated by multiplying the number of men by the length of time spent hunting and the mean horsepower of the vessels (Table 7, col. *D*). This was then prorated to the total kill to provide an estimate of the total standard effort expended by all components of the hunt (Table 7, col. *F*). The total effort was then partitioned into that component expended in the hunting of 1+ animals according to the ratio of the 1+ kill to the total kill (Table 7, col. *G*). This partitioning assumes that the pup kill is directly proportional to the hunting effort; this is a reasonable assumption since patch density of pups is independent of pup production (Sergeant 1971). The above analyses indicate that with the introduction of quotas in 1972 hunting effort on 1+ animals dropped to about 60% of the 1966–71 level and has shown a slight but consistent decline since 1974.

AGE-SPECIFIC HUNTING SELECTIVITY FACTORS

The selectivity pattern of the commercial harvest may be obtained by comparing the population age-structure with the catch age-structure. A representative population age-structure may be derived from samples of molting males providing adjustments can be made for the increased availability and vulnerability of young bedlamers (1- and 2-yr-olds). Fortunately empirical estimates of these biases are available from Sergeant (1976b) and these have been applied to age composition data of molting males in 1974 (Benjaminsen and Øritsland 1975a) to obtain a representative population

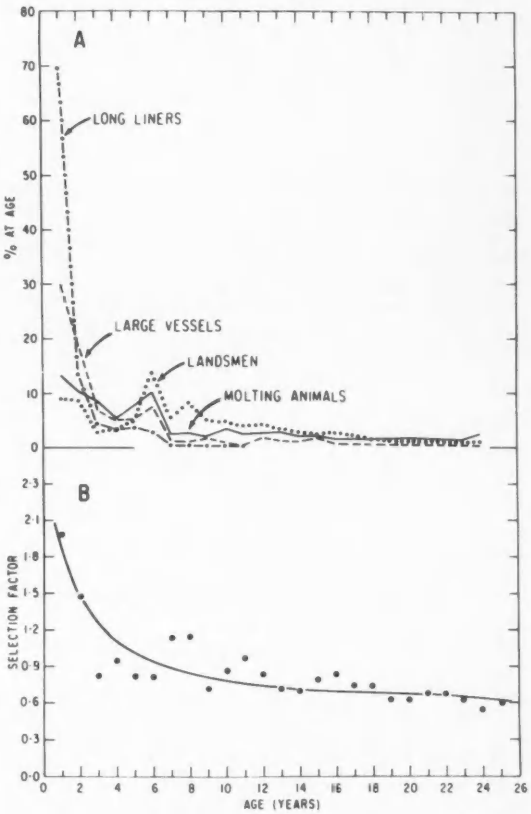

FIG. 1. Age compositions of harp seals harvested in 1974 by the major hunting components in relation to the population age structure (A) and the weighted selection factors thereby derived (B).

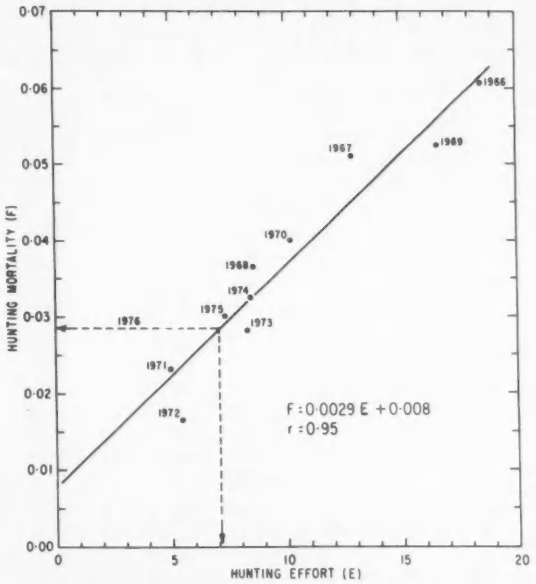

FIG. 2. Relationship between hunting mortality estimated from cohort analyses ($M = .10$) and hunting effort based on Norwegian kill statistics from the "Front."

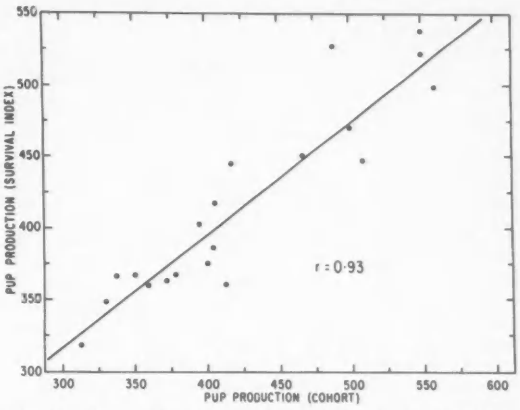

FIG. 3. Comparison of pup production estimates by cohort analyses and the Survival Index Method.

age-structure for that year. This is compared with the age-structure of the catch by the various hunting components in Fig 1A. The longliner harvest (vessels 11–21 m) is almost exclusively composed of bedlamers, whereas the large vessel (>21 m) kill is spread over the entire age range although a preference for young bedlamers is also evident. The landsmen kill tends to underselect bedlamers, concentrating instead on the intermediate age range of adults. The age composition of the total kill (weighted by the various components) expressed in terms of selection factors (Fig. 1B) indicates a predominance of bedlamers and a progressive underrepresentation of the older age-groups.

Sequential Computation of Production and Population Size

Estimates of harp seal population size and pup production may be derived from cohort analyses providing data are available that allow calculation of hunting mortality (F) in the most recent (terminal) year (1976) for which complete catch-at-age and effort data are available. One estimate of hunting mortality in 1976 (F_T) may be derived from the exploitation rate in that year. Thus the 1976 1+ kill of 36 000 animals from a population of 1.25 million (Table 6) represented an exploitation rate of .029 equivalent to $F = .031$. Hunting mortality in 1976 was also calculated from a functional regression of F on hunting effort for the period 1966–73, taking into account the goodness of fit of the 1974 and 1975 data points (Fig. 2). This gave an estimate of $F_T = .028$. An average value of $F_T = .030$ was selected and cohort analysis was run for the period 1952–76 with $M = 0.12$ (1952–60) and $M = 0.10$ (1961–76). From catch-at-age data (Lett and Benjaminsen 1977), population sizes were projected to 1978.

The results (Table 8) indicate that the harp seal population has declined from about 2.5 million animals in the early 1950s to <1.1 million in the early 1970s but has been increasing at an instantaneous rate of 0.03/yr since the introduction of quotas in 1972 and is now estimated to be around 1.3 million animals. Estimates of pup production by cohort analyses agree remarkably well with estimates from the Survival Index Method (Fig. 3) and confirm the validity of the natural mortality rates used.

Calculation of Front and Gulf Pup Production

The number of pups available to landsmen along northeast Newfoundland depends upon the "Front" pup production and the escapement from that production after the large vessel "whitecoat" harvest. Thus, utilizing a method proposed by Lett et al. (1977), a regression of catch/man on the large vessel pup kill at the "Front" should produce an inverse relationship with the intercept on the independent axis representing the average production on the Front for the period under consideration. Regression analyses, when applied to ordered pairs of Front large vessel kills and landsmen's catch rates for the period 1965–77 (1969 is excluded as being anomalous — see later discussion) (Fig. 4), indicate an average Front production of 211 000 pups. The average large vessel kill during this period was 109 000 animals resulting in an average escapement of 102 000 pups. Since escapement and catch/

TABLE 8. Age-specific estimates of harp seal population size from cohort analyses for the period 1952–78. (M = .12, 1952–59, M = .10, 1960–78)

	Population size ('000) at age											
Year	0	1	2	3	4	5	6	7	8	9	10+	Total (1+)
1952	551	247	290	191	188	155	222	113	122	91	851	2470
1953	557	315	219	247	162	161	131	186	92	100	789	2402
1954	485	320	260	189	214	141	140	113	163	79	764	2383
1955	554	265	250	218	164	185	122	121	94	141	679	2239
1956	610	262	210	195	187	141	160	105	104	79	671	2114
1957	501	263	218	179	169	162	123	138	89	88	620	2048
1958	466	293	208	182	152	144	139	105	119	76	575	1993
1959	510	282	216	164	149	123	119	115	87	99	526	1880
1960	419	237	225	180	139	127	109	102	98	77	498	1792
1961	414	231	179	187	153	117	108	93	88	85	487	1728
1962	395	216	203	159	166	135	105	97	83	79	506	1749
1963	404	166	169	151	135	142	116	92	85	72	503	1631
1964	411	107	143	145	129	117	124	100	79	73	482	1499
1965	350	127	95	126	125	110	101	105	87	68	450	1394
1966	361	144	108	82	109	107	92	84	92	77	454	1349
1967	371	123	119	88	70	93	91	77	71	80	454	1266
1968	400	82	101	103	78	61	81	78	66	62	452	1164
1969	378	220	68	87	90	69	54	72	68	57	445	1230
1970	335	128	179	59	76	79	59	46	63	59	429	1177
1971	327	103	108	155	51	66	69	52	40	55	423	1122
1972	306	103	86	96	138	45	59	62	47	36	412	1084
1973	337	168	92	76	84	123	39	52	55	41	399	1129
1974	332	212	150	81	67	74	108	34	46	49	377	1198
1975	326	193	183	131	71	59	65	95	30	41	377	1245
1976	316	169	164	161	116	62	52	57	84	26	367	1258
1977	320	165	142	142	142	102	54	46	50	75	350	1268
1978	338	172	144	124	124	125	91	48	41	45	385	1299

TABLE 9. Calculation of harp seal production on the "Front" from estimates of escapement from the large vessel kill. Total production estimates are from cohort analyses; Gulf production has been obtained by simple subtraction of "Front" from total production.

Year	Front escapement	Large vessel pup kill	Front production	Total production	Gulf production	% Front
1965	143280	63910	207190	350000	142810	59
1966	81270	172220	253490	361000	107510	70
1967	12040	184330	196370	371000	174630	53
1968	111670	85900	197570	400000	202430	49
1969	153210	176000	329210	378000	48790	87
1970	114980	134530	249510	335000	85490	74
1971	75250	133440	208690	327000	118310	64
1972	60500	104110	164610	306000	141390	54
1973	143580	63370	206950	337000	130050	61
1974	78560	85400	164050	332000	167950	49
1975	110170	109830	220000	326000	106000	67
1976	149300	93940	243240	316000	72760	77
1977	130030	80550	210580	320000	109420	66
Mean	104910	114430	219340	343000	123740	64

man should be linearly related through the origin, the ratio mean escapement/mean catch rate can be used to predict escapement which when summed with the large vessel kill also provides an estimate of Front production. Since the average catch/man is 3.4,

$$\text{Escapement} = 30.1 \times \text{Catch/man}.$$

Pup production at the "Front" has tended to fluctuate without trend during the past decade (Table 9), reflecting in part the relatively small changes in total pup production during this period but also reflecting variations in the proportion of the breeding population whelping at the Front as opposed to the Gulf of St. Lawrence. The unusually large production on the Front

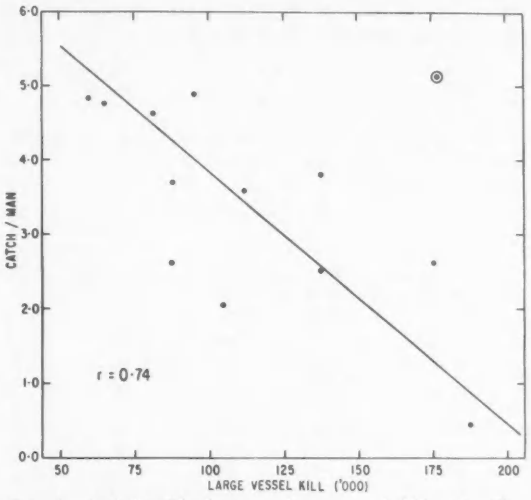

FIG. 4. Relationship between large vessel kills of whitecoats at the "Front" and subsequent landsmen kill rates along northeastern Newfoundland 1965–77. The circled point (1969) has been ommitted as being anomolous (see text).

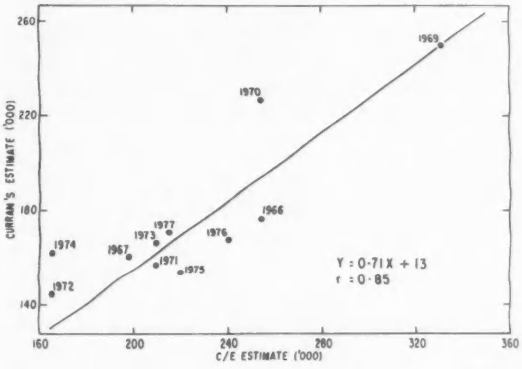

FIG. 5. Comparison of "Front" pup production estimates by the visual method (Curran's) and the catch/effort method 1966–77.

in 1969 resulted from sparse ice formation in the Gulf area that year; this forced a large percentage of the Gulf population to migrate northwards through the Strait of Belle Isle in late February which eventually congregated on standing local ice in the Groswater Bay area where they pupped in a relatively small area (T. Curran, Fisheries and Marine Service, St. John's, Nfld., personal communication). From catch statistics and aerial surveys Sergeant (1971) estimated the 1969 production in the Gulf to be 40 000 pups compared with 49 000 animals estimated by catch/effort analyses in Table 9.

The relative contribution of the Front production to

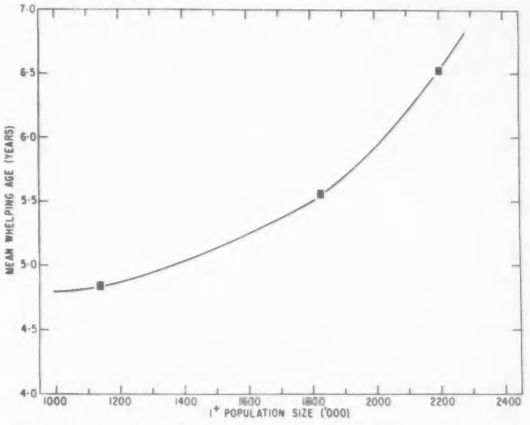

FIG. 6. Relationship between mean whelping age of Northwest Atlantic harp seals and the 1+ population size for the period 1952–76.

total production has fluctuated from 49 to 87% since 1965, the average of 64% being very similar to the value of 61% obtained from mark–recapture experiments (Sergeant 1977b). These fluctuations suggest some interchange between Front and Gulf adults thus reducing the danger of overexploitation of the Front population which currently accounts for about 75% of the total kill.

Harp seal production on the Front has been estimated annually since 1966 from direct aerial surveys by Mr Tom Curran, Chief Protection Officer in charge of sealing operations for Newfoundland using the method described by Lett and Benjaminsen (1977); Mr Curran's estimates are compared with those from catch/effort data in Fig. 5. The estimates agree remarkably well, the visual method being on the average 20% below the estimate from catch/effort data. Considering Mr Curran's claim that his visual estimates are always conservative by at least 10–15%, the above results pay fitting homage to the experience and acuity of a gentleman who has devoted most of his service to the conservation and management of Atlantic seal herds.

LOGISTIC CHANGES IN MATURATION AND FERTILITY RATES

Sergeant (1966) reported a reduction in mean whelping age of harp seals from 6.5 to 5.5 yr in the early 1960s following a period of heavy exploitation, which according to Sergeant (1966) elicited a density-dependent response from the seal population. A more recent estimate of the mean whelping age indicates a further decline to 4.7 yr (Sergeant 1976b). These data have been plotted against the 1+ population size (5-yr running average with 1-yr lag for the year of parturition) in Fig. 6, assuming that juvenile growth (and hence maturation rate (Laws 1959)) is stock de-

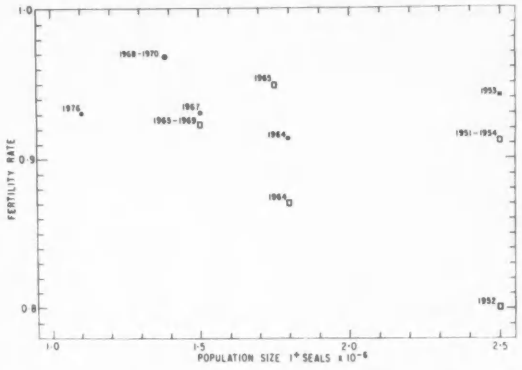

FIG. 7. Relationship between fertility rate of harp seals and 1+ population size. □ = Gulf; ● = Front.

pendent. The results indicate an exponential decrease in whelping age as the population declined from high levels in the early 1950s to relatively low levels in the early 1970s. This is in variance with the assumption by Lett and Benjaminsen (1977) of a linear shift in maturity ogive and implies a more rigid constraint on the population in periods of high and increasing abundance.

Lett and Benjaminsen (1977) have proposed that the fertility rate (proportion of reproductively successful females) of harp seals has also shown a density-dependent response since the early 1950s. Their conclusion is based on a plot of fertility rate on population size for the period 1952–76, but unfortunately no discrimination was made between data points from the Gulf and Front. Fertility rate data (all mature age-groups inclusive) are replotted in Fig. 7 for the Gulf and Front separately. With the exception of the 1952 value (80%) there is no discernible trend in the fertility rate since the early 1950s. The 1952 sample (Fisher 1952) was taken in the northern Gulf of St. Lawrence in January when normally the younger adults are mainly represented (Sergeant 1971) and these tend to have lower fertility rates than older females (Øritsland 1971). The 1952 value would therefore appear to be anomalous and this is supported by the 1953 sample from the Front and the combined 1951–54 sample from the Gulf, both of which are in the general range of 90–95% successful pregnancies. Given the uncertainties related to small sample sizes and varied seasonal sampling times from which the fertility rate estimates were derived, a logistic response in fertility rate cannot yet be categorically demonstrated for Northwest Atlantic harp seals.

Surplus Production (Sustainable Yield) of Pups

If R denotes the ratio of adult female recruits to pup survivors (i.e. $R = \frac{e^{-z}}{2}$), A denotes annual adult mortality, and F the fertility rate, the surplus production (sustainable yield rate (%)) of pups may be defined as

$$S.P. = \frac{FR - A}{FR}.$$

Surplus production of pups under various combinations of whelping ages and mortalities are illustrated below ($M = .10$, $F = 92\%$).

Whelping age (yr)	R: Pup kill only (A)	R: Universal kill (B)	Surplus production (sustainable yield): (A)	Surplus production (sustainable yield): (B)
4	.335	.291	68	59
5	.303	.255	64	53
6	.274	.222	60	47
7	.248	.194	56	39
8	.225	.170	52	30
10	.184	.130	41	9
12	.150	.100	28	0
13	.136	—	15	—
14	.120	—	0	—
Adult morality (Z)	.10	.115		
Bedlamer mortality (Z)	.10	.135		

Since whelping age of harp seals is a logistic function of population size, the above table suggests that changes in maturation rate profoundly affect the rate of surplus production and hence effective regulation of population size. The table also indicates that in the absence of density-dependent changes in natural mortality the harp seal population of the Northwest Atlantic has a whelping age of 14 yr at maximum equilibrium (i.e. virgin) population size. Such a drastic change in maturation rate does not appear likely (Sergeant 1969) implying density-dependent mortality probably at the whitecoat stage. It is not possible to evaluate this hypothesis from the data available since the commercial pup harvest probably acts to obscure and replace such a logistic response.

Pup Production and Reproductive Stock

The attrition in the harp seal population from the early 1950s to the early 1970s produced concomitant effects on pup production; the magnitude of the change, however, is ameliorated somewhat by reduced hunting on adult females and logistic changes in maturation rate. Thus a plot of pup production per unit reproductive stock (recruitment rate) versus reproductive stock (females only to avoid confounding effects of changing sex ratios) should provide an estimate of the change in net productivity of the population since the early 1950s. The results (Fig. 8A) indicate that a significant increase in the recruitment rate of harp seals has occurred in response to declining population levels, implying a non-linear stock–recruit relationship (Fig. 8B). The

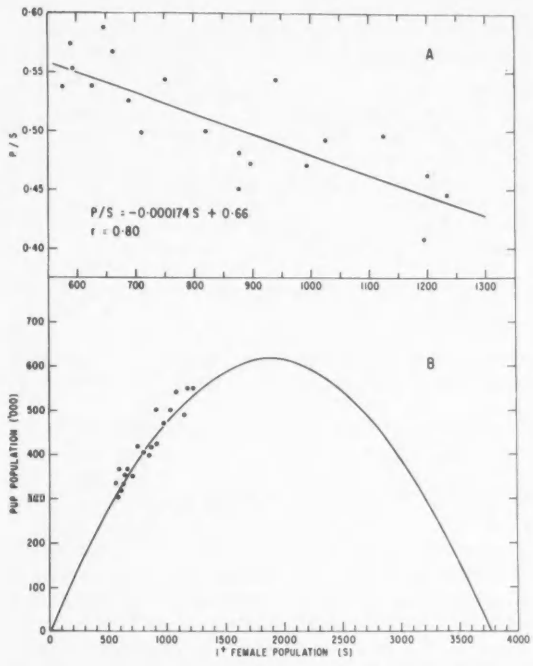

FIG. 8. Relationship between harp seal recruitment rate P/S and reproductive stock (1+ females) (A) and (B) between production and reproductive stock. ● = Pup production estimates from cohort analyses.

Graham–Schaefer type curve thus derived indicates a maximum equilibrium production of 615 000 animals occurring at a population size of 1.8 million 1+ females equivalent to a total 1+ population size of 3.6 million animals which is an estimate of the aboriginal population level of Northwest Atlantic harp seals. An indication of the aboriginal pup production of this herd may be obtained from maximum kills during the beginning of large-scale commercial hunting in the early 19th century. The largest kill reported was 687 000 animals in 1844 (Chafe et al. 1923). This represented the harvest by Newfoundland vessels only (i.e. excluded landsmen) but included both harp and hooded seals as well as animals older than 0-group. Taken together and assuming that the pup kill was nearly equivalent to the total production suggests a pup production of the order of 650 000 animals. Actual pup production in individual years may have been greater or less than 615 000 animals in the aboriginal state depending upon the efficacy of the response of reproductive rate to population change.

Maximum Sustainable Yield of Northwest Atlantic Harp Seals

The above information describes the Northwest Atlantic harp seal population as having a natural mortality

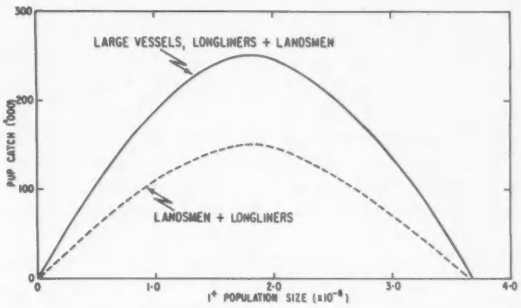

FIG. 9. Graham–Schaefer type yield curves for Northwest Atlantic harp seals. See text for explanation.

rate (M) of 0.10 and a maximum equilibrium stock size of 3.6 million seals producing 615 000 pups. Such information combined with the stock–recruit model (Fig. 8) and the density-dependent whelping model (Fig. 6) (extrapolated to 14 yr at 3.6 million seals) allows calculation of maximum sustainable yields as generated by the average hunting mortality and selection pattern on 1+ seals during the period 1975–77 when all three hunting components contributed significantly to the total kill. The results (Fig. 9) indicate a maximum sustainable yield of 230 000 pups and 60 000 1+ animals assuming that the present pup to 1+ kill ratio (80% pups) is maintained. The MSY population is 1.8 million animals with a breeding stock of 460 000 females.

A similar estimate of MSY has been made considering a harvest by landsmen and longliners only. This model assumes a nondiscriminatory kill of adults, selection factors weighted by the relative kills of the two components, and a pup to 1+ kill ratio of 60% (average 1975–77). The MSY under these conditions is predicted to be 150 000 pups (Fig. 9) and 100 000 1+ animals for a total yield of 250 000 animals.

From 1952 to 1972 when quotas were imposed the harp seal population of the Northwest Atlantic declined from approximately 2.5 million animals to 1.1 million animals (Table 8), i.e. approximately 70 000 animals (1.4 million/20) in excess of the yield required to stabilize the population at the level in the early 1950s. The average kill over this period was 300 000 seals, indicating a sustainable yield of 230 000 animals under equilibrium conditions. Since the harp seal population was above the MSY population size in the early 1950s, 230 000 is a minimum estimate of MSY under kill conditions as they existed during the 1950s and 1960s (pup to 1+ kill ratio of 70% and average 1+ hunting mortalities of approximately 0.05). Another estimate of MSY is the long-term kill. During the period 1830–1977 the average kill was approximately 275 000 animals and since the population was not at the size of maximum productivity for the entire time period, this estimate may also be considered a minimum estimate of MSY.

Discussion and Conclusions

The maximum sustainable yield of Northwest Atlantic harp seals harvested according to recent hunting patterns is estimated in this paper to be 290 000 animals (80% pups) from a 1+ population of 1.8 million animals producing 460 000 pups annually. This estimate is substantially higher than the MSY estimate by Lett and Benjaminsen (1977) of 240 000 animals from a 1+ population of 1.6 million animals producing 375 000 pups annually. The differences are mainly due to the extra constraint on population productivity exerted by the density-dependent fertility function included in Lett and Benjaminsen's (1977) model; in addition their higher natural mortality estimate ($M = .114$) implies a lower sustainable yield rate. Critical examination of fertility rate data in this paper fails to provide substantive support for any change in this parameter since the early 1950s and combined with a new, more rigorous estimate of natural mortality suggest that the MSY estimate of Lett and Benjaminsen (1977) is somewhat conservative. This conclusion is supported by recent and historical kill statistics providing minimum estimates of MSY in the range 230 000–275 000 animals.

In the last several years favorable ice conditions at the Front combined with an expanding and more efficient longliner fleet have resulted in a substantial increase in the harp seal harvest from that area. During the period 1975–77 pup kills at the Front averaged 125 000 animals which (from Table 9) represents an exploitation rate of 55.5%. This is above the present sustainable yield rate (53%) and raises the possibility of local overexploitation of Front production, assuming that Front and Gulf whelping herds are discrete. This stock relationship hypothesis has been discussed in detail by Sergeant (1965, 1970, 1971, 1977a), the conclusion being that while bedlamer seals tend to mix randomly between the Front and Gulf herds there is some degree of homing in the mature age-groups. This homing tendency does not appear to be intractable as evidenced by the fluctuating contributions of Front production to total production (Table 9). In particular, recent recoveries of two Gulf-tagged adults at the Front (D.E. Sergeant personal communication) and the diversion of most of the Gulf breeding population to the Front in 1969 (Sergeant 1971; T. Curran personal communication) support the conclusion that homing in the adult population is a facultative rather than obligatory aspect of harp seal behavior. Thus moderate overexploitation of the Front population is probably sufficiently diffused into the total population to avoid serious effects on Front production, particularly since bedlamers that experience the highest hunting mortalities among the 1+ age-groups are randomly mixed.

Cohort analyses (Table 8) and forward projections from the Survival Index Method (Table 6) suggest that the harp seal population of the Northwest Atlantic has been increasing at an instantaneous rate of 0.03 per year since the introduction of quotas in 1972. While long-term projections of population trends tend to be obscured by stochastic variability (Lett and Benjaminsen 1977), short-term projections can provide useful information on the effect of particular harvesting levels. Figure 10 illustrates the effect on population size and productivity in the period 1978–83 of a 180 000 kill distributed over the age-groups according to recent hunting patterns. Both pup production and population size (1+) are predicted to increase at an instantaneous rate of 0.02 /yr under these conditions implying that the MSY population size of 1.8 million animals would not be achieved until the mid-1990s. The sustainable yield at present levels of pup production (335 000 animals) is calculated to be 220 000 animals (80% pups), substantially above the 1978 quota of 180 000 animals; this is consistent with present management policies (Anon. 1977, 1978) designed to allow the harp seal population to increase gradually towards the MSY level.

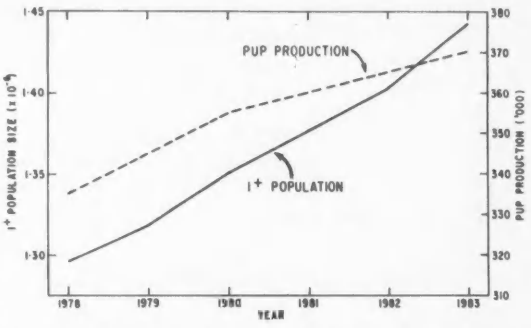

FIG. 10. Projection of pup production and 1+ population size of harp seals for the period 1978–83 under the conditions described in the text.

Acknowledgments

The development of this paper over the past several years has been made possible through contributions of data, ideas, and critical appraisals by a wide variety of scientific and management personnel. I therefore thank P. Lett, D. Sergeant, T. Øritsland, T. Curran, T. Benjaminsen, Ø. Ultang, D. M. Lavigne, and W. E. Ricker.

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Rapid Method for Determining Concentrations of Bayer 73 in Water During Lampricide Treatments

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DAWSON, V. K., P. D. HARMAN, D. P. SCHULTZ, AND J. L. ALLEN. 1978. Rapid method for determining concentrations of Bayer 73 in water during lampricide treatments. J. Fish. Res. Board Can. 35: 1262–1265.

Two simple, rapid, sensitive methods were developed for determining the concentration of the lampricide 2′,5-dichloro-4′-nitrosalicylanilide (Bayer 73) in stream water. Bayer 73 was extracted from acidified water samples with chloroform and then hydrolyzed to 2-chloro-4-nitroaniline (CNA) with either acid or base. The CNA was diazotized with sodium nitrite, and an azo dye was formed with either *N*-(1-naphthyl) ethylenediamine dihydrochloride (after acid hydrolysis) or 1-naphthol (after base hydrolysis). There was no interference from the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) in either method. Standard curves were prepared with untreated water to compensate for interfering substances that occurred naturally in some streams. The methods were sensitive to about 0.005 mg/L (ppm). Time required for analysis of a sample ranged from 25 min to 1 h.

Key words: Bayer 73, Bayluscide®, 2′,5-dichloro-4′-nitrosalicylanilide, lampricide, phenylamide, analysis, water, colorimetric, sea lamprey, Great Lakes

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Les auteurs ont mis au point deux méthodes sensibles, rapides et simples de dosage du lampricide 2′,5-dichloro-4′-nitrosalicylanilide (Bayer 73) dans de l'eau de rivière. Ils ont extrait au chloroforme Bayer 73 d'échantillons d'eau acidifiée et ensuite hydrolysée à 2-chloro-4-nitroaniline (CNA) soit avec un acide, soit avec une base. Ils ont ensuite diazoté le CNA avec du nitrite de sodium et il s'est formé un colorant azoïque avec l'un ou l'autre de *N*-(1-naphthyl)éthylénédiamine dihydrochlorure (après hydrolyse à l'acide) ou de 1-naphthol (après hydrolyse avec base). Il n'y a pas d'interférence de la part du lampricide 3-trifluorométhyl-4-nitrophénol (TFM) dans l'une ou l'autre des méthodes. On a préparé des courbes étalons avec de l'eau non traitée afin de contrebalancer les substances gênantes qui se rencontrent naturellement dans certains cours d'eau. Les méthodes sont sensibles à environ 0.005 mg/L (ppm). L'analyse d'un échantillon prend de 25 min à 1 h.

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THE selective lampricide 3-trifluoromethyl-4-nitrophenol (TFM) and the 2-aminoethanol salt of 2′,5-dichloro-4′-nitrosalicylanilide (Bayer 73) are used in combination (98% TFM, 2% Bayer 73) to control sea lampreys in tributaries of the Great Lakes. The combination was reported to be more effective and less costly than treatment with TFM alone (Howell et al. 1964).

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Direct colorimetric analysis can be used to monitor in-stream concentrations of TFM during treatments, but the method is not sensitive enough to measure Bayer 73 (Smith et al. 1960). Strufe (1962) reported a method for analysis of Bayer 73 in water, but it is not sensitive enough for determination of lampricidal concentrations. Daniels et al. (1965) reported methods for analysis of TFM and Bayer 73 in water, but background interference precludes its use for analysis of Bayer 73 at the concentrations used for lamprey control.

Although El-Dib and Aly (1972) reported a sensitive method for the determination of phenylamide pesticides in water, their method is too time consuming to be practical for field use. Our purpose was to develop a simple, rapid, sensitive method for determining Bayer 73 in stream waters.

Materials and methods — Two techniques were developed, procedure A involving acid hydrolysis and B, base hydrolysis. Both are simple, rapid, and sensitive enough for monitoring concentrations of Bayer 73 during stream treatments. In procedure A, acid hydrolysis is followed by color development with *N*-(1-naphthyl)ethylenediamine dihydrochloride (NED). In procedure B, base hydrolysis is followed by color development with 1-naphthol.

We analyzed equivalent concentrations (based on active ingredient) of Bayer 73 (99%), Bayer 73 (70% wettable powder), Bayer 2353 (Bayer 73 minus the aminoethanol salt), and CNA by both procedures to evaluate possible interferences due to formulation differences. Also, potential interferences resulting from the presence of TFM, phytoplankton, or suspended particulate matter in stream waters were examined.

Both methods were evaluated during an actual stream application of TFM and Bayer 73 on the Cedar River and its tributaries in Michigan during May 1976. The reagents and apparatus needed for each method, and the detailed directions for each, follow.

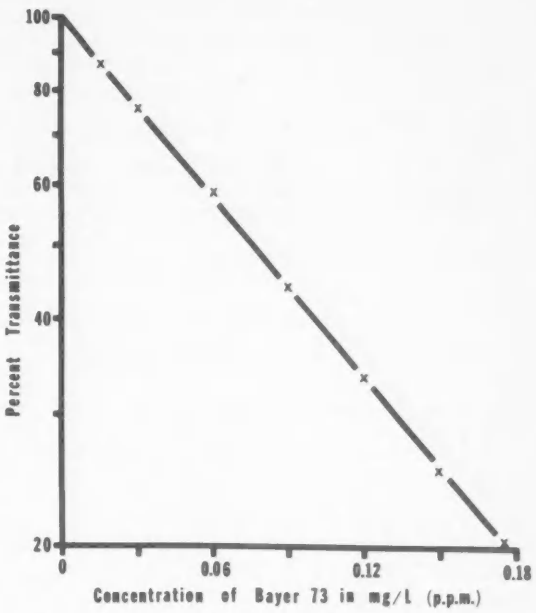

FIG. 1. Typical Bayer 73 standard curve in distilled water.

Procedure

A and B Chloroform.

A and B 2% sodium nitrite in distilled water. (Make solution fresh daily. Precalibrated spoons may be used for measuring reagents in the field.)

A and B 10% sulfamic acid in distilled water. (Make solutions fresh daily.)

A and B 2-aminoethanol salt of 2',5-dichloro-4'-nitrosalicylanilide (Bayer 73): transfer 10 mg of Bayer 73 to a 100-mL volumetric flask and bring up to volume with reagent grade methanol (use for preparation of standard curve; make solution fresh weekly and store in the dark.)

A and B 500-mL separatory funnels, 1-mL and 10-mL pipettes, 15-mL graduated Pyrex test tubes with Teflon-lined screw caps, warm-water bath, vortex stirrer, spectrophotometer, and spectrophotometer cuvettes.

A 0.2% *N*-(1-naphthyl)ethylenediamine dihydrochloride (NED) in distilled water. (Make solutions fresh daily.)

A Concentrated sulfuric acid, reagent grade.

B 10 N (28%) sulfuric acid.

B 2 N sodium hydroxide.

B 0.2% 1-naphthol in methanol.

Procedure A

1. Prepare standard curve (similar to Fig. 1) by spiking 300 mL samples of untreated stream water with Bayer 73 from the stock solution to achieve a series of concentrations in the range of the expected treatment concentration (0.15 mL yields 0.05 mg/L).

2. Add 10 mL of concentrated H_2SO_4 to 300 mL of water sample and standard solutions in 500-mL separatory funnels.

3. Add 7 mL of $CHCl_3$ and shake vigorously, opening stopcock frequently to release pressure.

4. Drain $CHCl_3$ fraction into 15-mL graduated screw-capped test tube and retain aqueous fraction. Evaporate $CHCl_3$ (in uncapped tube) in warm-water bath with stream of dry air (in hood or well-ventilated area).

5. Repeat extraction steps (3) and (4) on the aqueous fraction and evaporate $CHCl_3$ fraction to dryness. Discard aqueous fraction.

6. Add 1 mL of concentrated H_2SO_4 to each sample and to a blank. Cap tightly and place in boiling water bath for 5 min.

7. Cool and dilute each tube slowly (addition of water to concentrated sulfuric acid generates heat and causes spattering) to 7 mL with distilled water.

8. Cool and add 1 mL of $NaNO_2$ to each tube, cap, and shake. Allow 1 min for complete diazotization.

9. Add 1 mL of sulfamic acid to each tube, cap, and shake for at least 1 min.

10. Add 1 mL of developer (NED) to each tube, cap, and shake.

11. Transfer contents of test tubes to cuvettes. Use reagent blank to set spectrophotometer to 100% transmittance at 535 nm. Insert cuvettes containing samples and read percent transmittance. Refer to standard curve to determine milligrams per litre (parts per million) of Bayer 73 present.

Procedure B

1. Prepare standard curve (similar to Fig. 1) by spiking 300 mL samples of untreated stream water with Bayer 73 from the stock solution to achieve a series of concentrations in the range of the expected treatment concentration (0.15 mL yields 0.05 mg/L).

2. Add 30 mL of 10 N H_2SO_4 to 300 mL of water sample and standard solutions in 500-mL separatory funnels.

3. Add 7 mL of $CHCl_3$ and shake vigorously, opening stopcock frequently to release pressure.

4. Drain $CHCl_3$ fraction into 15-mL graduated screw-capped tube and retain aqueous fraction. Evaporate $CHCl_3$ (in uncapped tube) in warm-water bath with stream of dry air (in hood or well-ventilated area).

5. Repeat extraction steps (3) and (4) on the aqueous fraction and evaporate $CHCl_3$ fraction to dryness. Discard aqueous fraction.

6. Add 1 mL of 2 N NaOH to each sample and to a blank. Cap tightly and place in boiling water bath for 5 min.

7. Cool, add 0.25 mL of 10 N H_2SO_4 to each tube and mix.

8. Add 0.25 mL of $NaNO_2$ to each tube and mix. Allow 1 min for complete diazotization.

9. Add 0.25 mL of sulfamic acid to each tube and mix for at least 1 min.

10. Add 0.25 mL of 1-naphthol to each tube and mix.

11. Add 1 mL of 2 N NaOH to each tube.

12. Dilute each tube to 10 mL with distilled water; cap and shake.

13. Transfer contents of test tubes to cuvettes. Use reagent blank to set spectrophotometer to 100% transmittance at 590 nm. Insert cuvettes containing samples and read percent transmittance. Refer to standard curve to determine milligrams per litre (parts per million) of Bayer 73 present.

Results and discussion — Two colorimetric methods were developed for the analysis of Bayer 73 in stream water. The methods compare favorably in sensitivity; however, the choice of the method used will be based on background interferences in the stream and the laboratory facilities available to the analyst. Method A utilizes concentrated acid hydrolysis and color development with *N*-(naphthyl)ethylenediamine dihydrochloride to form a red color with maximum absorption at 535 nm. Method B uses a milder base hydrolysis and color development with 1-naphthol to form a blue color with maximum absorption at 590 nm. The absorbance maximum for method B is farther upscale than that for method A and as a result has less potential for color interferences. Method B requires an additional pH adjustment during color development; however, reagent volumes are designed for use of micropipettors, which facilitate reagent additions.

An analytical method for field use must give rapid and efficient recovery of Bayer 73. Chloroform was selected as the extracting solvent for both procedures because its relatively high specific gravity allows it to be drawn off the bottom of a separatory funnel.

Because the amount of extraction solvent must be kept small to reduce the time required for evaporation, we determined solvent quantities and extraction efficiencies for chloroform by using ^{14}C-Bayer 73. Recoveries ranged from 95 to 108% when 2 times 5-mL volumes of chloroform were used for extraction of 200-mL water samples or 2 times 7-mL volumes for 300-mL water samples.

Although El-Dib and Aly (1972) used 50% H_2SO_4 for hydrolysis, we found that Bayer 73 was not completely hydrolyzed with 25, 50, or 75% H_2SO_4 after 5 and 10 min. However, Bayer 73 was completely hydrolyzed to 2-chloro-4-nitroaniline (CNA) in concentrated H_2SO_4 (procedure A) or in 2 N NaOH (procedure B) when heated in a boiling water bath for 5 min. Thin-layer chromatography confirmed the formation of CNA in the hydrolysis procedures.

Walker and Schoettger (1967) used 0.2% $NaNO_2$ to form the diazonium salt of MS-222® but this procedure required 15 min. Our procedures use the 2% $NaNO_2$ recommended by El-Dib and Aly (1972), and trials at 0.5, 1.0, 1.5, and 2.0 min indicated no increase in dye formation for diazotization reactions longer than 1.0 min. El-Dib and Aly (1972) allowed 10 min for the destruction of excess nitrite with sulfamic acid, but laboratory studies ranging from 0.5 to 10 min indicated that 1 min of mixing completely eliminates excess nitrite.

The reaction of the developer NED (procedure A) with primary aromatic amines was described by Bratton and Marshall (1939). The literature suggests that the azo dye thus formed is light sensitive and requires 5–10 min for complete formation. Laboratory studies showed that the azo dye formation is rapid and that it is not light sensitive, possibly because the medium in our method is highly acidic. The use of an acidic medium throughout procedure A eliminates steps requiring changes in pH, and NED was used in this procedure because its azo dye can be formed in an acidic medium. However, the use of concentrated H_2SO_4 and subsequent dilution can be hazardous, especially under field conditions. Color development in procedure B involves the use of 1-naphthol, as recommended by El-Dib and Aly (1972).

We found that filter colorimeters such as the Klett-Summerson equipped with filter #54 (procedure A) or filter #59 (procedure B), or grating spectrophotometers were suitable for color quantitation. A battery-operated Bausch and Lomb Spectronic Mini 20 spectrophotometer is acceptable for on-site analysis.

Time required for the analysis of a sample by either procedure ranged from 25 min to 1 h, depending on the familiarity of the technician with the procedures. The limit of sensitivity of both methods is about 0.005 mg/L of Bayer 73. Use of a mechanical vortex stirrer and an autopipette greatly increases the speed and ease of sample analysis.

Both procedures produced similar results when equivalent concentrations (based on active ingredients) of Bayer 73 (99%), Bayer 73 (70% wettable powder), Bayer 2353 (Bayer 73 minus the aminoethanol salt), CNA, CNA plus TFM, and CNA plus chlorosalicylic acid were used. The mean and standard deviation for the percent recoveries resulting from this comparison was 98.9 ± 6.5, indicating no interference from ingredients used in formulation.

Laboratory analysis of Bayer 73 in water containing TFM, phytoplankton, or suspended particulate matter resulted in recoveries of 101, 100, and 100%, respectively. However, large quantities of plankton in environmental samples may produce formation of troublesome emulsions during extraction.

One problem was encountered with both procedures during the on-site evaluation of the methods during

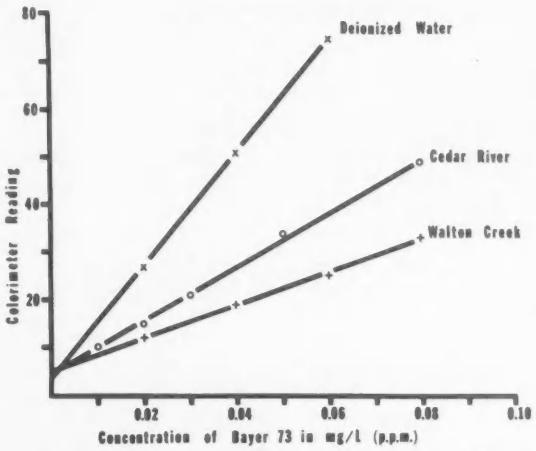

FIG. 2. Bayer 73 standard curves from three different water sources (procedure B).

treatment of the Cedar River, Mich. Spiked samples of water from the Cedar River and one of its tributaries, Walton Creek, produced different standard curves, both of which indicated lower recoveries than a comparable standard curve using deionized water. The interferences from these streams were observed using both procedures. Typical standard curves resulting from one of the procedures is presented in Fig. 2. However, when the standard curve prepared from the water collected from a given stream the day before chemical application was used for monitoring that stream's chemical treatment, correction of the lower recoveries was achieved, and the measured peak concentrations of Bayer 73 in the streams approximated levels expected on the basis of chemical application rates and measured stream flow rates. Four replicates gave a mean and standard deviation of $100 \pm 1.9\%$ recovery. The presence of high concentrations of tannins and lignins in some streams may cause a shift in the standard curves for some stream waters. Stream water from each treatment site should be used in the preparation of standard curves.

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Lag Adjustment Between Estimated and Actual Physiological Responses Conducted in Flow-Through Systems

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NIIMI, A. J. 1978. Lag adjustment between estimated and actual physiological responses conducted in flow-through systems. J. Fish. Res. Board Can. 35: 1265–1269.

The results from physiological experiments that use a flow-through or open system may be biased because of lag. The nature of this discrepancy is attributable to a delay between the actual response of an organism and the measured response of the system. An equation is presented that would adjust the measurement for lag. This procedure is especially important when measuring time-dependent uptake and depuration rates like those reported in respiratory and toxicological studies.

Key words: lag, flow-through systems, open systems, time-dependent uptake, depuration rate, respiration, toxicology

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Il est possible que les résultats d'expériences physiologiques dans un système à flot continu ou ouvert soient biaisés à cause d'un retard dans les réponses. Cette différence est attribuable au retard qu'il y a entre la réponse effective d'un organisme et la réponse mesurée du système. Nous donnons une équation qui apporterait à la mesure une correction tenant compte de ce retard. Cette procédure a une importance spéciale dans la détermination des taux d'assimilation et de dépuration dépendant du temps, comme ceux que l'on mentionne dans des études de respiration et de toxicologie.

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CERTAIN physiological measurements on aquatic organisms may require a flow-through or open type of apparatus to ensure that experimental conditions can be regulated. The advantages of such a system are partially offset by an apparent need to adjust the results for a differential time interval between the response of an organism and a concurrent measurement. This difference has been termed lag (Fry 1971). While compensation for lag may not always have been a common practice in investigations using an open system (Northby 1976), its effects can be particularly significant in studies that examine time-dependent relationships such as uptake and depuration rates. This exercise reviews the nature of lag, examines its effects on measurements, and suggests an adjustment procedure.

Specifically, lag is the time interval between the response of an organism and the response of the system. Duration of the lag, and magnitude of the estimated response are dependent on the volume of a system and its flow rate. A ratio of the volume/flow rate can be termed the turnover rate (T). For example, a T of 3.0 would describe a system with a capacity of 30 L and a flow of 10 $L \cdot h^{-1}$. Decreasing the flow rate would increase the T value for the system. Similarly, flow rates for systems of differing capacities can be adjusted to the same T value.

The significance of lag can be addressed by reviewing selected examples of oxygen consumption measurements reported for fishes. Three types of responses generally observed (Fig. 1) include a constant level of oxygen uptake such as active metabolism (Fry 1957), an exponential change as the time required to attain a specific level (Beamish 1964), and the "cost of feeding" (Muir and Niimi 1972). The third is the most complex type of response and can be examined with reference to the total amount of oxygen required to utilize a meal (Beamish 1974) and the shape or profile of the curve.

In a static or closed system, consumption rates have been estimated by multiplying the difference in oxygen content of water before and after a time interval by its volume, whereas in an open system the difference in oxygen content between the inflow and outflow is multiplied by the flow rate (Stroganov 1962). Values derived from open systems using this procedure may be referred to as unadjusted estimates because no compensation is made for lag. In retrospect, the protocol for estimating respiratory rates using a closed system appears to be accurate, while estimates derived from an open system may not totally account for residual oxygen in the chamber, the flow, or influence of the turnover factor.

The estimated response would also be affected by the time interval between introduction of the organism and the start of measurements. In some experiments, an organism respiring at a constant rate may be maintained in an open system long enough to establish equilibrium oxygen concentrations between the inflow and outflow before measurements commence. In other experiments, measurements may be taken immediately after introduction of the organism into the system. This precondition must be taken into account.

Estimates of a constant rate of uptake measured in an open system, but whose computations do not account for lag, would be accurate provided measurements are continued until a quasi-steady state has been attained (Fig. 1, IA). In practice, the response of an organism that sharply increased its respiratory rate from 10 to 30 mg $O_2 \cdot h^{-1}$ would not be immediately monitored because of lag. Theoretically it would require 3 h to approach within 95% of the expected level in a system with a T value of 1.0, 8 h at a T of 3.0, and 13 h at a T of 5.0. Where an adjustment for lag is applied, an estimate of the actual respiratory rate would be almost immediately available, the limiting factors being the analytical precision and the time interval between measurements.

Where a response to a specific metabolic level is being measured, for example, an exponential decrease in respiratory rate from 30 to 10 mg $O_2 \cdot h^{-1}$, spurious estimates of an organism's response can be expected in an open system because of the difference between an apparent response and the actual response that is at-

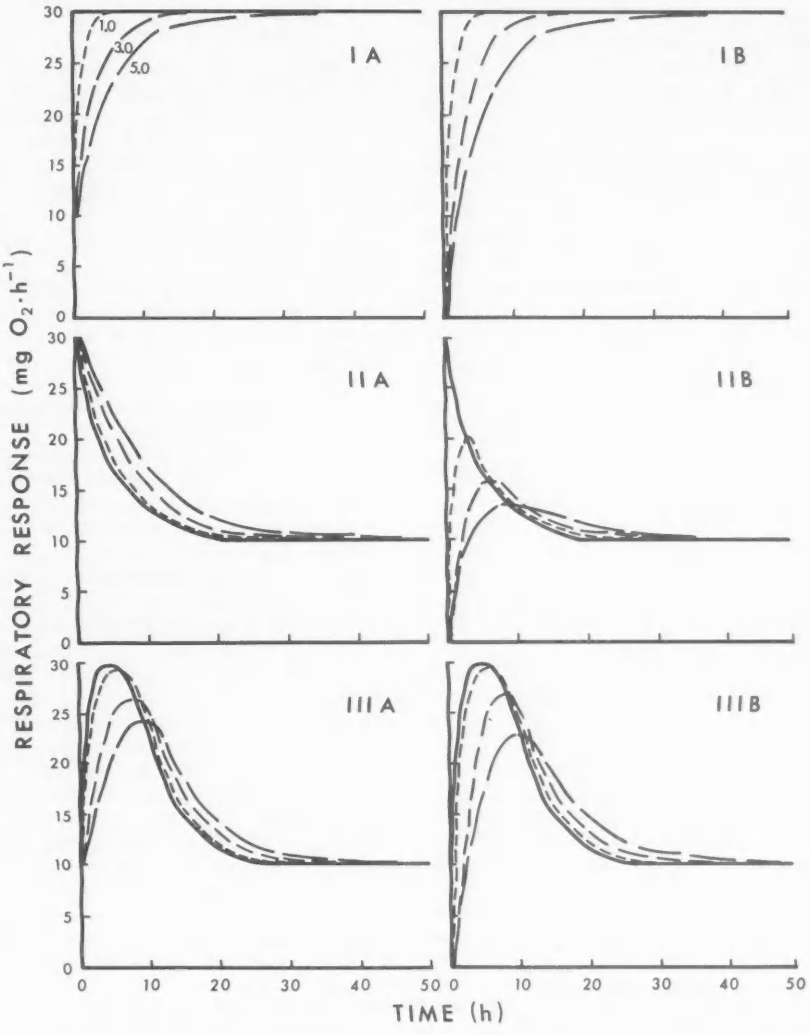

FIG. 1. Effect of lag on estimated rate of oxygen uptake demonstrated by fishes for the constant level (I), exponential change (II), and cost of feeding (III) types of responses. Solid lines represent the theoretical respiratory response of the organism, and broken lines the unadjusted response estimated from systems with volume/flow ratios (T) of 1.0, 3.0, and 5.0. The type A responses represent estimates for systems where the inflow and outflow concentrations are in equilibrium with the organism before measurements commence, and the type B responses those estimates where measurements are taken immediately after introduction of the organism into the system.

tributable to lag. Failure to adjust this type of response for lag would provide poor estimates of the rate of and time to attain a specific level. Errors of this nature will increase proportionately with increasing T values as the magnitude of this difference is increased.

A measure of the cost of feeding is the most difficult type of response to assess because of rapid changes in respiratory rates over a relatively short time interval (Fig. 1, IIIA). This curve will follow a respiratory pattern of increase, perhaps reaching a plateau, and then a decrease. An accurate measure of the total respiratory requirement, indicated by the area under the curve, would be possible without adjustment for lag where oxygen measurements are continued until a quasi-

steady state condition; however, failure to adjust these estimates for lag would provide erroneous values of the maximum rate of oxygen uptake, duration of elevated uptake, and shape of the response curve. Errors of this nature become more pronounced with increasing T values.

The effect of establishing equilibrium conditions within a system before measurements commence is best demonstrated by the unadjusted estimates of a type II response where the curves generally follow the response of the organism. Where equilibrium conditions have not been established, the shape of the curves differs from the response of the organism (Fig. 1, IIB). This effect is less pronounced for a type I response where only the time to reach a steady state is prolonged. For a type III response, an unadjusted estimate would show a lower maximum rate of uptake, and the shape of the curve would be shifted to the right, the same effect demonstrated by increasing T values.

The interpretation of unadjusted estimates can be particularly difficult when an organism has established only partial equilibrium before measurements begin. The response curve will assume a position between the type A and B curves, and its shape would depend on the degree of equilibrium attained. While its effect on a type I curve would be minimal, it would continue to provide spurious estimates for a type II response although the curves may better approximate the actual response of the organism. It could also affect an estimate of the total respiratory requirement of a type III response. It would be a relatively simple procedure to estimate the area under the curve at equilibrium conditions where the baseline of the pre- and postelevated levels are similar (Fig. 1, IIIA). Where complete equilibrium conditions have not been established (Fig. 1, IIIB), the total oxygen requirement can still be accurately measured provided the baseline follows a curvilinear relationship. The nature of its curvilinearity is affected by the T value and the degree of equilibrium established. In view of these difficulties of data evaluation on measurements conducted in open systems, an adjustment procedure for lag would be advantageous.

The following equation will adjust the three types of responses described for lag irrespective of whether equilibrium conditions have or have not been established before measurements are taken.

$$\Delta X/\Delta t = \frac{FB\, e^{-\frac{F}{V}(t_1-t_0)} - FC}{1 - e^{-\frac{F}{V}(t_1-t_0)}} + FA$$

where X represents the amount of material,
t the time,
F the flow rate,
V the volume of the chamber,
A the concentration of the material in the inflow at t_0,
B the concentration of the material in the outflow at t_0, and
C the concentration of the material in the outflow at t_1.

The mathematical expression of this equation is more detailed than the adjustment procedure suggested by Fry (1971); however, the proposed equation would provide a better measure of the actual response particularly in open systems monitoring type II and III responses at high T values. Where T values are low, and the difference in concentration between the two outflow measurements is small, either adjustment equation would be satisfactory.

Measurements of excretion or depuration rates conducted in open systems can also be adjusted for lag according to this equation. It would be most readily applicable to those measurements such as the excretion of body metabolites and the depuration of pollutants that are released at measurable quantities over a relatively short time interval. In contrast to uptake measurements, the equation would yield a negative value to suggest more material is leaving the system than is entering.

The equation was derived from the laws of growth and decay and the principles of displacement time of molecules where the concentration of a material would change exponentially by a coefficient of 0.693, in accordance with first order kinetics equations, each time a volume of water equal to its capacity has flown through the system. The concept of displacement time has been discussed by Sprague (1969), and the coefficient by Atkins (1969). Following these premises, the one assumption that was made was a uniform mixing in the chamber whereby the concentration of the material at the outflow would be the same as that found in the chamber.

To test the accuracy of this equation, the unadjusted response at different T values (Fig. 1) was calculated from contrived data according to the following procedure. Using a constant oxygen concentration at the inflow, and accounting for a theoretical rate of uptake by an organism, the oxygen concentration at the outflow was calculated by prorating the decrease of residual oxygen at hourly intervals by a coefficient of 0.693 each time a volume of water equal to the capacity of the chamber has flowed through. The unadjusted values were calculated by multiplying the difference in oxygen concentrations by the flow rate (Stroganov 1962). The same levels of oxygen concentrations estimated at hourly intervals at the outflow was then applied to the proposed equation. The adjusted estimates agreed to well within $\pm 1\%$ of the theoretical value.

To support the application of this equation for lag adjustment, an artificial system was devised that would simulate the response of an organism (Fig. 2). With a 1- or 2-L cylindrical chamber, and a series of peristaltic pumps to achieve constant flow rates, an open system where theoretical responses at different T values could be manipulated was possible. To simulate the response of an organism, a second inflow system, modified to allow for systemic dilutions and concentrations by

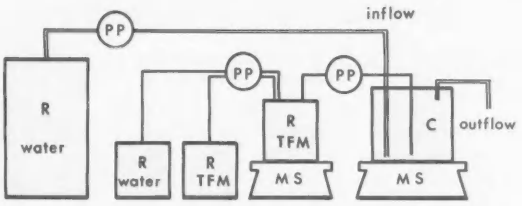

FIG. 2. Design of apparatus used to simulate the response of an organism in an open system. Flow rates from the reservoirs (R) containing water and TFM were regulated with peristaltic pumps (PP), and thorough mixing in the chamber (C) was achieved by using a magnetic stirrer (MS).

using multichannel persistaltic pumps in series, was used to release predetermined concentrations of 3-trifluoromethyl-4-nitrophenol (TFM) into the chamber. A magnetic stirrer was used to achieve thorough mixing. By varying the concentrations of TFM, and the rate of introduction, portions of the different types of theoretical responses could be simulated and measured. Concentrations of TFM were determined colorimetrically according to Olson and Marking (1973). Using this procedure, it was possible to examine several conditions of the equation. TFM concentrations in the two chambers decreased by a coefficient of 0.691 ± 0.002 (SE) for 18 measurements at T values of 1.0–5.0. This coefficient is consistent with the hypothesis. Simultaneous measurements taken from different areas within the chamber and outflow indicated identical concentrations of TFM which would support the assumption of uniform mixing. Sequential measurements at the outflow provided the values necessary to corroborate the theoretical responses. This equation was also demonstrated to be equally applicable to depuration measurements.

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Precocious Sexual Maturation in Atlantic Salmon (*Salmo salar*) Postsmolts Reared in a Seawater Impoundment

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SUTTERLIN, A. M., P. HARMON, AND B. YOUNG. 1978. Precocious sexual maturation in Atlantic salmon (*Salmo salar*) postsmolts reared in a seawater impoundment. J. Fish. Res. Board Can. 35: 1269–1272.

Female Atlantic salmon, *Salmo salar*, usually become sexually mature following a minimum of one winter at sea. Between 4 and 16% maturation was observed for both sexes in two different stocks of salmon placed in sea water as 1+ yr smolts and subsequently reared one summer. Only the larger females and the smaller males matured. Egg size and fertility appeared normal. Smoltification at an early age or accelerated growth rates may be responsible for this phenomenon.

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Les saumons atlantiques, *Salmo salar*, femelles atteignent ordinairement la maturité sexuelle après un minimum d'un hiver en mer. On a observé entre 4 et 16% de maturation chez les deux sexes dans deux stocks différents de saumons placés dans de l'eau de mer comme smolts d'âge 1+ et maintenus dans ce milieu durant un été. Seuls les grandes femelles et les petits mâles atteignirent la maturité sexuelle. La taille des oeufs et la fertilité semblèrent normales. Il est possible qu'une smoltification hâtive ou un rythme de croissance accéléré soit responsable de ce phénomène.

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PRECOCIOUS sexual development in male Atlantic salmon (*Salmo salar*) underyearlings and parr is commonly observed under natural and hatchery conditions (Saunders and Henderson 1976; Saunders and Sreedharan 1977). Less common is the occurrence of sexually mature male postsmolts in salt water (Menzies 1914; Hutton 1960; Shearer 1963; Saunders and Henderson 1965). A small percentage appeared during saltwater tank-rearing studies by Saunders and Henderson (1965), and in estuarine pens by Naevdal et al. (1975). In the latter case, the mature males were presumed to have originated from those male parr that had not smoltified sufficiently. Least common is the occurrence in nature or during saltwater pen-rearing of sexually mature female salmon after only 5–6 mo in salt water. Lee and Power (1976) recorded appreciable egg development in two smolts migrating from Leaf River, Ungava Bay. An accelerated rearing regime, producing large, 1+ yr smolts, is described that resulted in an appreciable proportion of both males and females maturing after one summer in an outdoor saltwater impoundment. An attempt has been made to explain this anomaly based on an altered presmolt history. These observations were parts of a study designed to assess the suitability of a variety of salmonid species and hybrids for saltwater culture.

Materials and methods — The stock origin and freshwater rearing conditions for the two groups of Atlantic salmon — landlocked (LL) and searun (SR) — have been described previously (Sutterlin et al. 1977). Following hatching, the two groups were reared at low density under an increasing temperature and photoperiod regime. By October, 10 mo posthatching, the fish had reached 20 g in weight and had become silvery, resembling smolts. Their salinity tolerance was considerably higher than parr but had not reached the level usually accepted for a true smolt, i.e. tolerance of 40‰ salinity for 100 h (Komourdjian et al. 1976; Sutterlin et al. 1977). After October, both lots were maintained in brackish water (15% and 16°C) with 20 h of light per day until June 1977. They were then gradually acclimated to sea water over a period of 3 wk then placed together in a large saltwater tidal impoundment (15 × 30 × 7 m), and reared for a further 5 mo. While in sea water the two groups (numbering 118 LL; 197 SR) were fed twice daily to satiation on homemade moist pellets. Temperature in the impoundment ranged from 10°C in June to 16°C in August, then down to a minimum of 6°C in November when the fish were removed. The majority of these fish were sacrificed in mid-November and a sample was retained in freshwater until spawning.

Results — After 5 mo in salt water, 4–6% of the males and 8–16% of females in the groups approached spawning condition (Table 1). The testis weight for maturing males in mid-November averaged between 6 and 9% of body weight for the two groups. In a sample of females held in freshwater until they became ripe in January, fecundity determined by hand stripping averaged between 1000 and 1300 eggs per kilogram body weight for the two groups. Egg diameter varied between 4–5 mm. Seven of the 12 females stripped produced viable eggs that eyed properly following fertilization while the eggs produced by the remaining females had improper yolk distribution, possibly re-

TABLE 1. Growth and sexual maturation following 5 mo of salt water rearing of sea-run (SR) and landlocked (LL) salmon.

	LL salmon	SR salmon
Number sampled	100	100
In salt water June 23, 1977		
length (cm)	21.6	21.8
weight (g)	112.1 (June 7)	120.6
Out salt water Nov. 15, 1977		
length (cm)	29.6	30.4
weight (g)	301.1	327.3
Growth rate % wet wt / day	0.61	0.69
% survival	94	97
% maturing		
males	4	6
females	8	16
Testes wt / body wt × 100 (Nov. 15)	6.1	9.3
Egg yield kg (Jan. 10)	1039	1266

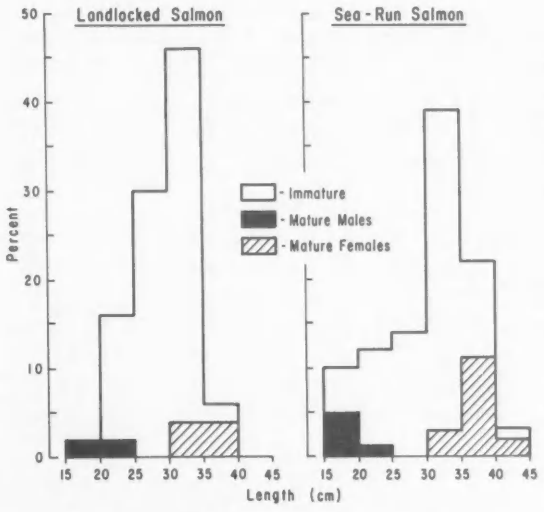

FIG. 1. Size distribution of sexual mature and immature salmon in November.

sulting from delayed spawning. Maturation was related to body size in that only the larger females in the sample had well developed eggs (Fig. 1); the ovaries of the remaining females comprised less than 1% of their body weight. Maturing females were still silvery at the time of egg taking and had not developed the yellow-brown hue typical of spawning females having spent one or more years at sea. Ripe males on the other hand had developed a golden brown hue, but possessed no hooked lower jaw typical of male grilse and larger ripe males. Milt produced by these males was capable of fertilizing salmon ova.

Discussion — Among the multitude of factors influencing the onset of sexual maturation in Atlantic salmon, age, growth rate, seasonal factors, and genetic propensity are usually considered to be most important.

Although various Atlantic salmon stocks reared under the same conditions differ considerably in age at first maturity (Naevdal et al. 1975, 1978; Saunders and Sreedharan 1977), it is doubtful that the precocious sexual maturation described here is entirely due to a peculiarity in the strains chosen. More probably the early fresh or brackish water rearing regime somehow altered the maturation process. The sea-run salmon were obtained from Middle Brook, Nfld., which is predominantly a grilse river. Several years of brood stock collections from the lake supplying the landlocked salmon used in this study indicate that the size at maturity in the lake is not radically different from the outcome observed in both stocks of fish used here. Landlocked females first mature at a body weight of less than 1 kg and mature males can be found weighing only 100 g. Mature females, however, are usually 5–6 yr old.

Most wild smolts in eastern Canada enter the sea at a weight of approximately 30 g. For optimum returns, it is customary for hatchery smolts to be released at a somewhat larger size (40+ g). During this study, the "smolts" were placed in brackish water somewhat earlier than is usual for wild or hatchery smolts and they were placed in full sea water at a much larger size (110–120 g).

The increasing photoperiod and temperature conditions during the freshwater and brackish rearing phase might have been responsible for accelerated maturation. Although photoperiod manipulation has been clearly shown to influence the smoltification process in Atlantic salmon (Knutsson and Grav 1975; Komourdjian et al. 1976), the use of yearling parr in their studies and underyearlings in ours complicates a comparison of results. In our study, either the production of smolt-like fish in the fall after only 10 mo rearing in freshwater or the early introduction into brackish water would have permitted another entire year for female gonad development to occur by the following fall.

Sea-run salmon returning as mature female grilse are seldom under 1.3 kg in weight, while those females maturing during this study weighed between 0.5 and 0.7 kg. The fact that most mature females were the largest in the lot suggests that they were probably larger smolts, initially having reached critical size to commit themselves to the maturation process. Results with hatchery smolts of 2+ age released in the wild by Ritter (1972) are in part opposite the results reported here, in that his larger smolts matured later. Sex differences, however, were not examined by Ritter (see Simpson and Thorpe 1976).

The size of mature males removed from sea water in this study tended to be the smaller fish in both lots. During monthly weighings of fish throughout the freshwater rearing phase, no instances of "ripe or running" male parr or smolts were observed. This does not exclude the possibility that some of the males were in advanced stages of maturation when they were placed in sea water, resulting in poor subsequent growth and regression to a parr characteristic, such as annual sexual maturation (Evropeytseva 1960). Retention of males in freshwater past smoltification also results in the same regressive process (Evropeytseva 1962).

Recent attempts to reduce the production costs of Atlantic salmon smolts, have focused on the feasibility of producing a smolt in less than 10 mo from hatching. Environmental, genetic, and hormonal manipulations are being considered. Smolts would therefore be available in late summer or fall for releasing or stocking in fish pens in countries where ocean temperature permits. Although growth that winter would result in a large postsmolt the following spring, one possible detrimental result might be a high incidence of small mature females appearing the following fall.

Acknowledgments — Thanks are extended to R. H. Peterson, E. B. Henderson, and R. L. Saunders who provided valuable criticisms of the manuscript.

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Adaptation to Environment: Essays on the Physiology of Marine Animals. *Edited by R. C. Newell. Butterworth & Co. Ltd., London — Boston. 1976, 539 p. $59.50.*

This publication, as its title implies, is not simply a textbook on the environmental physiology of marine organisms, but is a collection of essays by leading scientists in their own respective fields of physiological endeavor. The reader will not find this volume a comprehensive review of the entire subject of physiological adaptation of marine organisms. The editor however, has skillfully assembled a series of essays 'each of which is intended as a review of special adaptations of marine organisms to the particular environmental conditions which they are likely to encounter in the natural habitat.' As Dr Newell points out, this approach has led to differences in style and presentation technique among the contributing authors, however, this does not detract from the work, as virtually all presentations are of good quality and highly readable.

Subjects covered include intertidal and estuarine life adaptation, settlement responses in marine organisms, biochemical adaptations to temperature pressure, and low oxygen, primitive respiratory adaptations, rhythmic behavior and reproduction in marine organisms, and vision in pelagic animals. Contributing authors include R. C. Newell, D. J. Crisp, G. N. Somero, P. W. Hochachka, C. P. Mangum, A. P. M. Lockwood, E. Naylor, and J. K. Bowmaker. Somero and Hochachka have made a major contribution to the volume in the form of three separate essays on biochemical adaptations to temperature, pressure, and low oxygen. A wealth of information is summarized for the student of both vertebrate and invertebrate physiology.

There are several features that I particularly liked about Adaptation to Environment. A major asset of the work is that so much of the information is up to date. As Dr Newell points out, emphasis has been placed throughout the text on recent developments in the subject and on new approaches to the study of the adaptations of marine organisms. Furthermore, a good subject and author index is provided which is often a weakness in compilations of this type. Adaptation to Environment is a useful addition to the literature. I have no hesitation in recommending it highly to interested readers.

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(R182)

Coelenterate Ecology and Behavior. *Edited by G. O. Mackie. Plenum Press, New York, N.Y., 1976, xiv + 744 p. $49.50.*

Of all the marine invertebrate phyla probably none has captured man's attention more than the coelenterates, with probably the most compelling reason being the sheer beauty of these often ephemerally delicate organisms, seemingly neither plant nor animal. It is this other aspect, their evolutionary place in the order of things, that makes them also one of the most important test organisms in today's laboratory. It is their place near the fork between the plant and animal kingdoms, their relative "simplicity" of cellular organization, and their ready intracellular and metabolic symbiosis with algal cells that has lifted this phylum from one of purely esthetic appreciation to a central position in modern cell research.

This shift in emphasis and their central focus in biochemical and molecular studies, more traditionally associated with the white rat or with *Drosophila*, is amply demonstrated in George Mackie's "Coelenterate Ecology and Behavior," the proceedings of the Third International Symposium on Coelenterate Biology held at the University of Victoria in 1976. The scope of the volume is broader than the title suggests, however, ranging from papers on the hydrodynamics of anemones to ultrastructural studies of epithelial intra-communication. As such, Mackie's book represents an update of the trends of current coelenterate research, not only in the phylum per se, but also more generally with respect to those fields of invertebrate neurophysiology and behavior, of intracellular symbiosis, and of functional morphology and ecology.

"Coelenterate Ecology and Behavior" has the appearance of a tome — not surprisingly since over 70 papers are included. As is to be expected from a symposium proceedings, each paper is pretty much a study onto itself, and any continuity there exists is largely the result of editorial grouping, with one exception, the section on neurophysiology (but more on that later). Papers are grouped into six general divisions — Water Flow in the Ecology of Benthic Cnidaria; General Ecology of Bottom-living Forms; Biology of Ctenophores, Siphonophores, and Medusae; Reproductive Biology; Associations, Functional Morphology; and finally Behavioral Physiology. Everybody in "coelenterology" who was or is anybody, with few exceptions, participated in the symposium and is represented in the volume.

Now as to my views on these proceedings. In retrospect I view the book with mixed feelings. The volume is sound, and Mackie's organization of the papers makes for a good blend, but for the most I missed those sparkling papers in which a time-honored theory is challenged, or where a novel new technique is applied to a traditional problem. Generally speaking, most of the papers presented add further information to a growing coelenterate literature, but there was little, for example, on the mind-boggling adaptations of zooxanthella to host animal tissues, or vice versa, or on the control of zooxanthella metabolism by host cellular machinery. "Self"-recognition in competitive interactions is well-recognized and documented but the precise molecular or biochemical basis is not understood, and there was nothing on this. That is not to say that there were no interesting papers. The introductory talks by Wainwright's group on the use of hydrodynamic principles in organism structure and support are definitely new, and introduce a new concept of biology. Impressive was the truly large-scale study by Reeve and Walter on ctenophora growth and predation in 15 meter high offshore cylinders. There was also the interesting work of Lesh-Laurie describing the possible role of DNA synthesis in regional stolon and hydranth determination in *Pennaria* planulae.

Although Muscatine did not contribute to this volume directly, his area of interest, that of intracellular symbiosis, is represented in four papers (Pardy, Kelly and Cook, Schoenberg and Trench). Of particular interest is the study by Schoenberg and Trench because of their use of the technique of single-linkage clustering of isozyme patterns in an attempt to unravel the puzzling specificity of zooxanthellae to a wide range of coelenterates.

The most potent section in this volume is, however, the one on behavioral physiology, and it could well serve as a mini-symposium on its own rights. It consists mainly of electrophysiological studies, including most of the "veterans" in this field — Morin, Passano, de Kruijf, Tardent, McFarlane, Mackie himself, and others. About the only one missing in this line-up is Bob Josephson. Papers in this section are a good mix of experimental electrophysiology and of ultrastructural studies, juxtaposing the process with the structure. It is hard to single out any one paper but this reviewer was drawn for personal reasons to "Mac" Passano's excellent and lucid thoughts on the coelenterate brain. It is in this last series of papers that the symposium, on reading, came to life. Maybe it is a reflection of George Mackie's own interests and his life-long preoccupation with ferreting out the nervous basis for an exceedingly beautiful and captivating organism, the coelenterate medusa, or maybe it is simply that the neuron boys are better writers.

Please don't take my somewhat lukewarm comments too hard. This book needs to be on every library shelf and in every invertebrate biologist's reference collection. It is a true up-date of the state of the art in a very important field. As for the neurophysiologists of this world, it is a must.

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Index to Scientific and Technical Proceedings. *Volume 1, January 1978. Philadelphia, Pa., Institute for Scientific Information. ISSN 0149-8088. 440 p. Annual Subscription $500.00.*

Papers published in conference proceedings are often difficult to retrieve using the usual abstracting and indexing services. In this new publication from the publishers of the *Science Citation Index* and *Current Contents*, we have a new key to this elusive form of literature.

The publishers state that they intend to index some 3000 conference proceedings in the scientific and technical field in the 12 monthly issues to be published in 1978. This is estimated to be a little under 50% of the total published in the year. Their selection procedures are stated as covering those "of greatest interest to the scientific community."

The issue received starts with detailed instructions for using the various indexes to best advantage. This is followed by a "category" or general subject index to the proceedings listed in the issue. They are indexed under a number of headings which include: Biology, Ecology, Fisheries (which is mentioned in the introduction but does not appear in the index of this issue), Marine Biology, and Zoology. Under Ecology I noted two proceedings of potential interest to fishery biologists.

The next section lists some 250 proceedings and their contents in much the same way as contents appear in *Current Contents*. Full bibliographic details are given along with prices and suppliers' addresses.

This section is followed by a "Permuterm Subject Index" to the individual papers. This index is similar to that appearing in some *Current Contents* and that in the *Science Citation Index*. Under the main heading "Fish" there are some 140 entries to papers from the two conferences previously noted and isolated papers from three other conferences. Under "Salmo" I found a paper from a conference on endocrinology. Under "Trout" two papers from conferences previously covered were listed.

Separate author, sponsor, meeting location, and corporate address indexes are found at the end of the issue.

The publisher offers to supply on their OATS (Original Article Tear Sheet) Service individual papers from some (a minority in this issue) of the proceedings.

In order to estimate the value of this new service compared with already established retrieval tools, I checked to see if any of the six conference proceedings containing papers on fish have been covered by *BioResearch Index*. The first four issues for 1978 have not listed any of these proceedings. Two of the conferences are published annually in series which are indexed — the 1976 conferences were both indexed in the April 1977 issue. I would therefore expect to see them covered very soon by *BioResearch Index*. Whether or not the other four conference proceedings will be covered in *BioResearch Index* I can only guess at this time, but I expect that perhaps two out of the four other conference proceedings will be indexed. If this is correct, and the review copy is a representative sample, it may be expected that during the course of a year some 20–30 conferences of potential interest to fishery biologists may be covered by this new service and not by others. Therefore this is another source which should be searched when making a thorough literature search.

The $500 annual subscription includes semiannual cumulations as well as the regular monthly issues. At this price, only the larger libraries will be able to afford to subscribe and, in view of the broad spectrum covered, few special libraries will be able to justify buying the title.

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Books received/Livres reçus

April/avril 1978

Yearbook of Fishery Statistics. Catches and Landings. *Vol. 42. Food and Agriculture Organization of the United Nations, Rome, 1977. 325 p.*

Yearbook of Fishery Statistics. Fishery commodities. *Vol. 43. Food and Agriculture Organization of the United Nations, Rome, 1977. 338 p.*

Index to Scientific and Technical Proceedings. *Vol. 1, January, 1978. Institute for Scientific Information, Philadelphia, Pa., 1978. 440 p. Annual subscription $500.00.*

Marine Ecology. Volume III. Cultivation, Part 2. *Edited by O. Kinne. John Wiley and Sons, New York, N.Y., 1977. xv + 579–1293 p. $82.80.*

An Annotated Bibliography of the Muskellunge, *Esox masquinongy* (Osteichthyes: Salmoniformes). *E. J. Crossman and Cheryl D. Goodchild. Life Sciences Miscellaneous Publications, Royal Ontario Museum, Toronto, Ontario, 1978. 131 p. $4.50.*

May/mai 1978

Fish Protein Concentrate: Panacea for Protein Malnutrition? *E. R. Pariser, Mitchel B. Wallerstein, Christopher J. Corkery and Norman L. Brown. The MIT Press, Cambridge, MA, and London, England. 1978. xix + 296 p. $23.95 (Can.)* (The study documents the rising and falling expectations of fish protein concentrate (FPC) as a solution to the world's protein food crisis.)

Biological Investigations of the Vostok Bay. *Transactions of the Academy of Sciences USSR, Far East Science Centre, Institute of Marine Biology, Vladivostok, No. 5, 1976. (In Russian).* (The papers of this volume deal with the geological history and hydrology of the Vostok Bay, characteristics of bottom communities and fauna.)

Power Plant Entrainment. A Biological Assessment. *Edited by J. R. Schubel and Barton C. Marcy, Jr. Academic Press, Inc., New York, NY. 1978. x + 271 p. $16.00 (U.S.)* (Primarily addressed to decision makers, the book examines entrainment problems associated with once-through cooling systems of power plants.)

Journal of Fish Diseases, Vol. 1, No. 1, January, 1978. *Editor: R. J. Roberts; Associate Editor: R. Wooten. Blackwell Scientific Publications, Oxford, England. 1978. iv + 136 p. $50.00/year (Can. and U.S.)* (The first issue of the only journal devoted to fish diseases which is printed in English. It contains both papers and book reviews.)

A Coastal Marine Ecosystem. Simulation and Analysis. *J. N. Kremer and S. W. Nixon. Springer-Verlag New York, Heidelberg, and Berlin. 1978. xi + 217 p. $32.00 (U.S.)* (This is No. 24 in the Publisher's Ecological Studies Series, and describes the development of a mechanistic simulation model of Narragansett Bay (Rhode Island, U.S.A.).

Microbial Ecology of a Brackish Water Environment. *Edited by G. Rheinheimer. Springer-Verlag New York, Heidelberg and Beerlin. 1977. xi + 291 p. $39.60 (U.S.)* (This is No. 25 in the Publisher's Ecological Studies Series, and is a companion to the above-mentioned No. 24. The book describes a 15-months' investigation of Kiel Bight (Baltic Sea), and the two books together cover studies of coastal marine ecosystems.)

Planning of Aquaculture Development — An Introductory Guide. *T. V. R. Pillay. Published for FAO by Fishing News Books Ltd., Farnham, Surrey, England. 1977. 71 p. £4.80.* (Although originally based on a round table discussion at a FAO/Norad meeting, the book is an expanded version covering the strategies for establishing aquaculture as a viable industry.)

COMING NEXT MONTH/À PARAÎTRE LE MOIS PROCHAIN

Lowering of serum thyroxine and triiodothyronine levels in yearling coho salmon, *Oncorhynchus kisutch*, by dietary Mirex and PCBs
J. F. Leatherland and R. A. Sonstegard

Effect of extended galvanonarcosis on behavior of rainbow trout, *Salmo gairdneri*, and channel catfish, *Ictalurus punctatus* **K. Curry and B. Kynard**

Effect of ozonation on microbial fish pathogens, ammonia, nitrate, nitrite, and BOD in simulated reuse hatchery water **P. J. Colberg and A. J. Lingg**

Parasitic mastitis in the Atlantic white-sided dolphin, *Lagenorhynchus acutus*, as a probable factor in herd productivity **J. R. Geraci, M. D. Dailey, and D. J. St. Aubin**

Notes

Helminths of the alimentary tract from a stranded herd of the Atlantic white-sided dolphin, *Lagenorhynchus acutus* **M. Beverly-Burton**

First evidence for even-year spawning pink salmon, *Oncorhynchus gorbuscha*, in Lake Superior **W. Kwain and J. A. Chappel**

Histopathological and hematological changes in mummichogs, *Fundulus heteroclitus*, infected by a pseudomonad **M. F. Li, G. S. Traxler, and S. Clyburne**

Status of *Exosphaeroma inornata* Dow and *E. media* George and Stromberg (Isopoda: Sphaeromatidae) with ecological notes **E. W. Iverson**

Capture probability: the role of zooplankter escape in the selective feeding of planktivorous fish **R. W. Drenner, J. R. Strickler, and W. J. O'Brien**

Stomach flushing: effectiveness and influence on survival and condition of juvenile salmonids **W. R. Meehan and R. A. Miller**

Toxicological comparison of natural and cultured populations of *Acartia tonsa* to cadmium, copper, and mercury **S. L. Sosnowski and J. H. Gentile**

RECENT PUBLICATIONS / RECENTES PUBLICATIONS

The number following each title in each series refers to the establishments listed at the end of the section.

Les nombres qui suivent les titres de chacune des séries désignent les établissements suivants à la fin de la section.

Recent Studies

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles on investigations published in other periodicals, which henceforth may be obtained in reprint form only from the author or originating establishment.

2495. KICENIUK, J. W., W. R. PENROSE, AND W. R. SQUIRES. 1978. Oil spill dispersants cause bradycardia in a marine fish. Mar. Pollut. Bull. 9: 42–45. (9)

2496. ROGERS, I. H. 1978. Environmental effects of terpenoid chemicals: A review. J. Am. Oil Chem. Soc. 55: 112A–118A. (3)

2497. HARA, T. J. 1977. Amino acids as olfactory stimuli in fish, p. 157–164. *In* 6th Int. Symp. Olfaction Taste VI, Paris, France, July 1977. (4)

2498. SAETHER, O. A. 1976. Two species of *Chaoborus* (Diptera: Chaoboridae) from Venezuela. Acta Biol. Venez. 9: 195–212. (4)

2499. WHITAKER, J., J. BARICA, H. KLING, AND M. BUCKLEY. 1978. Efficacy of copper sulphate in the suppression of *Aphanizomenon flos-aquae* blooms in praire lakes. Environ. Pollut. 15: 185–194. (4)

2500. FREEMAN, H. C., G. SHUM, AND J. F. UTHE. 1978. The selenium content in swordfish (*Xiphias gladius*) in relation to total mercury content. J. Environ. Sci. Health 13: 235–240. (8)

Récentes études

Jusqu'en 1970, ces articles étaient réimprimés, puis reliés dans un volume annuel d'études, comprenant deux parties. Les études de 1969, partie 2, incluant le n°1355, ont été les dernières à être reliées. Nous n'en faisons désormais plus la reliure ni la distribution. Nous continuons cependant de numéroter les articles et d'en établir des listes d'études sur des recherches mentionnées dans d'autres périodiques. On peut donc se les procurer, sous leur forme réimprimée seulement, de l'auteur ou du service dépositaire.

2501. HSIAO, S. I. C., D. W. KITTLE, AND M. G. FOY. 1978. Effects of crude oils and the oil dispersant corexit on primary production of arctic marine phytoplankton and seaweed. Environ. Pollut. 15: 209–221. (5)

2502. MACKINNON, C. N., AND E. M. DONALDSON. 1978. Comparison of the effect of salmon gonadotropin administered by pellet implantation or injection on sexual development of juvenile male pink salmon (*Oncorhynchus gorbuscha*). Can. J. Zool. 56: 86–89. (3)

2503. HUNT, J. J. 1978. Age, growth and distribution of silver hake *Merluccius bilinearis*, on the Scotian Shelf. Int. Comm. Northwest Atl. Fish. Sel. Pap. 3: 33–44. (6)

2504. MUIR, D. C. G., AND B. E. BAKER. 1978. A method for the routine semiquantitative determination of hydroxy-s-triazines in soils. J. Agric. Food Chem. 26: 420–424. (4)

Interpretive Articles Series

This new series commenced in 1966. The articles interpret work already published in primary journals. They appear at irregular intervals in various technical publications. Frequently an article essentially duplicates, condenses, or is based on an article in another series.

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399. CHADWICK, M., R. PORTER, AND D. REDDIN. 1978. Atlantic salmon management program Newfoundland and Labrador, 1978. Atl. Salmon J. 1: 9–15. (9)

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3rd International Conference on Ephemeroptera
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Fisheries and Marine Service
501 University Crescent
Winnipeg, Manitoba
R3T 2N6

conférence : une excursion d'avant-conférence dans le sud de l'Ontario (28 juin au 4 juillet 1979) et excursion d'après-conférence en Saskatchewan et en Alberta (10 au 17 juillet).

Pour plus de renseignements sur cette conférence, on est prié de s'adresser à

John F. Flannagan, convocateur
3e Conférence internationale sur les Ephemeroptera
Institut des eaux douces
Service des pêches et de la mer
501 University Crescent
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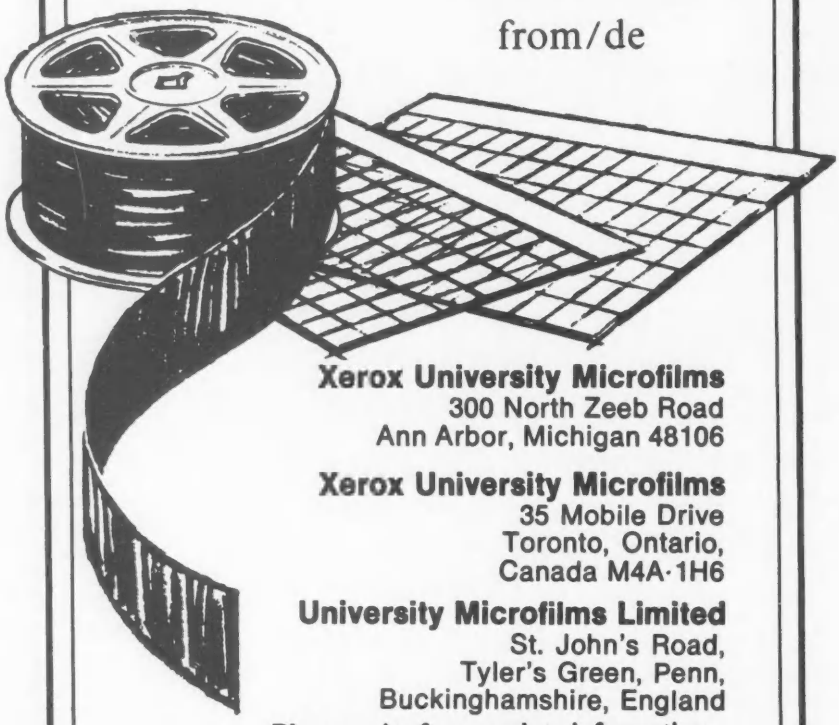

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SI Units and Symbols

We encourage use of SI units (Système international d'unités) and symbols in the *Journal of the Fisheries Research Board of Canada* for the following list of quantities. A more complete list is given in the *Canada Metric Practice Guide* (CAN3-Z234.1-76) available from the Canadian Standards Association, 178 Rexdale Blvd., Rexdale, Ontario M9W 1R3.

Unités et symboles SI

Nous encourageons l'usage des unités SI (Système international d'unités) et des symboles pour le *Journal de l'office des recherches sur les pêcheries du Canada* pour la liste des quantités ci-dessous. Le *Guide canadien de familiarisation au système métrique* (CAN3-Z234.1-76), que l'on peut se procurer auprès de l'Association canadienne de normalisation, 178, boul. Rexdale, Rexdale (Ontario) M9W 1R3, donne une liste plus complète des unités.

Quantity/Quantité	Name/Nom	Symbol/Symbole
length/longueur	centimetre, kilometre . . ./centimètre, kilomètre . . .	cm, km . . .
area/aire	square centimetre . . ./centimètre carré . . .	cm^2 . . .
volume/volume	millilitre, litre . . ./millilitre, litre . . .	mL, L . . .
time/temps	second/seconde	s
	minute/minute	min
	hour/heure	h
	day/jour	d
frequency/fréquence	hertz/hertz	Hz
mass/masse	gram, kilogram . . ./gramme, kilogramme . . .	g, kg . . .
density/masse volumique	kilogram per cubic metre/kilogramme par mètre cube	kg/m^3
pressure/pression	pascal/pascal	Pa
temperature/température	degree Celsius/degré Celsius	°C
electric current/courant électrique	ampere/ampère	A
conductance/conductance	siemen/siemen	S
luminous intensity/intensité lumineuse	candela/candela	cd
illuminance/éclairement	lux/lux	lx
amount of substance/quantité de matière	mole/mole	mol
molar concentration — volume/ molalité concentration — volume	mole per cubic metre/mole par mètre cube	mol/m^3
molar concentration — mass/ molalité concentration — massique	mole per kilogram/mole par kilogramme	mol/kg
ppm (parts per million)	microgram per gram/microgramme par gramme	μg/g
	milligram per litre/milligramme par litre	mg/L
	microlitre per litre/microlitre par litre	μL/L
ppb (parts per billion)	nanogram per gram/nanogramme par gramme	ng/g
	microgram per litre/microgramme par litre	μg/L

The following units are not acceptable in the SI and authors using them are asked to give SI equivalents. If both units are given it is preferable to put the non-SI one in parentheses.

Les unités qui suivent ne sont pas acceptées par le SI et les auteurs qui les utilisent sont priés de donner l'équivalent. Si les deux unités sont données, il est préférable de placer entre parenthèses celle qui n'est pas du système SI.

Not acceptable in SI/ Non valides dans le SI	SI equivalents/Equivalents SI	Conversion factors/ Facteurs de conversion
angström/angström (Å)	nanometre/nanomètre (nm)	1 Å = 0.1 nm
micron/micron (μ)	micrometre/micromètre (μm)	1 μ = 1 μm
calorie/calorie (cal)	joule/joule (J)	1 cal = 4.1868 J
inch (in)/pouce (po)	centimetre/centimètre (cm)	1 in/1 po = 2.54 cm
foot (ft)/pied (pi)	metre/mètre (m)	1 ft/1 pi = 0.305 m
mile/mille (mi)	kilometre/kilomètre (km)	1 mi = 1.6 km
curie/curie (Ci)	becquerel/becquerel (Bq)	1 Ci = 37 GBq

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